JOURNAL OF AGRICULTURAL RESEARCH

VOLUME 59

JULY 1–DECEMBER 15, 1939

ISSUED BY AUTHORITY OF THE SECRETARY OF AGRICULTURE
WITH THE COOPERATION OF THE ASSOCIATION OF
LAND-GRANT COLLEGES AND UNIVERSITIES

UNITED STATES
GOVERNMENT PRINTING OFFICE
WASHINGTON : 1940

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Published on the 1st and 15th of each month. This volume will consist of 12 numbers and the contents and index.

Subscription price:

Entire Journal: Domestic, $3.25 a year (2 volumes)
Foreign, $4.75 a year (2 volumes)

Single numbers: Domestic, 15 cents
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650.61
U58j
v.59
Loc.: pp 815-830

NOV 11 1940
442973

JOURNAL OF AGRICULTURAL RESEARCH

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AUTHOR'S ERRATA AND EMENDATION

Page 67, line 6, "532.94" should be "332.94."

Page 67, table 3, column 4, line 1, "407.20" should be "4076.20."

Page 68, line 15, "$(\bar{x}'-\bar{x}'')_{0.05}$" should be "$(\bar{x}'-\bar{x}'')_{0.05}$," and "$(\bar{x}'-\bar{x}'')_{0.01}$" should be "$(\bar{x}'-\bar{x}'')_{0.01}$."

Page 68, footnote 6, "$N_2=42$" should be "$N_1=42$."

Page 76, under heading, "Experiments with Other Aphids," lines 3 and 4, delete the words, "all of which were grown from seed."

Page 101, seventh line from bottom, "varieties of" should be "varieties or."

Page 105, tenth line from bottom, "north and south" should be "east and west."

Page 106, line 9, "longitudinal" should be "crosswise."

Page 112, line 11, "$-3Z_{\ldots w}$" should be "$+3Z_{\ldots w}$."

Page 116, lines 19 and 20, "$n=16$ ($n=n_1+n_2$ where n_1+1 and n_2+1 are each equal to 9)" should be "$n=5,104$ the number of degrees of freedom available for the estimation of error."

Page 117, line 17, "$\frac{\Sigma(X_{\ldots w})+\Sigma(Y_{u.w})+\Sigma(Z_{uv.})}{27}$" should be $\frac{\Sigma(X_{\ldots w})^2+\Sigma(Y_{u.w})^2+\Sigma(Z_{uv.})^2}{27}$."

Page 136, line 11, insert the word "regain" before "its."

Page 148, table 6, first word of heading "Sucrose," should be "Reducing sugars."

Page 203, table 5, headings, "Sum of square" should be "Sum of squares."

Page 385, first line, "S. Watts" should be "S. Wats."

Page 402, figure 3, magnifications should read A, B, ×65; C×60; D, E, ×1.

Page 481, table 5, column 1, "I" should be "IX."

Page 511, table 3, delete superscripts "3" from all data applying to leachings; for HPO_3,8,[1] middle pot, change superscript "1" to "2" for 1.78; for NaH_2PO_2 c. p., 4_1 middle pot, change superscript "2" to "3" for .66; for $Na_4P_2O_7$, c. p., 4^1 and $C_a(H_2PO_4)_2$ c. p., 4^1 top pot, change all six of superscripts from "1" to "2."

Page 595, figure 2, "32°" should be "30°."

Page 598, table 5, last column, the following figures should appear, from top to bottom: 54, 38, 42, 46, 75, 71, 43, 39, 36, 35, 68, and 75.

Page 657, legend for figure 9, "perfect" should be "imperfect."

Page 674, table 3, note under heading, "b=100 except where noted" should be "n=100 except where noted."

Page 679, figure 1, on both abscissa and ordinate "0.1" should be "0.01."

Page 693, table 5, the units for the last six columns from left to right are: "Percent, Percent, Number, Number, Percent, and Percent."

Page 743, line 18, "whereas with nitrate" should be "whereas with nitrite."

Page 760, line 15 of text, "its action on the fungus" should be "scandium's action on the fungus."

Page 777, ninth line from bottom, "5.66 for fat and 9.35 for protein" should be "9.35 for fat and 5.66 for protein."

Page 809, figure 1, last line of legend, "a and b more than strong plants c and d" should be "plant b more than strong plant d."

Page 859, table 1, next to last column, line 15, "1909–1934" for Fort Valley should be "1909–1930."

JOURNAL OF AGRICULTURAL RESEARCH

VOL. 59 WASHINGTON, D. C., JULY 1, 1939 No. 1

INFLUENCE OF MOISTURE SUPPLY ON DROUGHT RESISTANCE OF CONIFERS[1]

By HARDY L. SHIRLEY, *senior silviculturist*, and LLOYD J. MEULI, *formerly junior plant physiologist, Lake States Forest Experiment Station, Forest Service, United States Department of Agriculture*[2]

INTRODUCTION

In many regions of the United States, the success of forest plantings depends to a considerable extent upon the use of well-hardened, drought-resistant planting stock. Over extensive areas of the Western States, the Plains region, and the Lake States severe droughts are common, and only the more drought-resistant species and individuals can be depended upon to survive and make satisfactory growth. Even in regions normally well supplied with rainfall, hardy planting stock is required on the thin or badly eroded soils which are being planted to forest trees.

In northern Minnesota, the unusually dry summer of 1930 caused widespread damage to forest trees; in unfavorable locations, young trees 6 to 8 feet in height were killed (*14*).[3] Severe drought recurred in 1933, 1934, and 1936. Mortality in recently established forest plantations was high in all 4 years. The greatest damage occurred in 1936, when 50 percent of the seedlings newly planted on sandy soils were killed. The drought of 1936 was accompanied by a period of extremely high temperatures, which not only accentuated the moisture stress within the plants but critically injured many seedlings by overheating. Such losses emphasize the need of more exact knowledge of the factors influencing survival of trees during periods of drought, especially factors subject to human control.

Although field observations and biochemical tests have given us a general knowledge of the physiology of drought resistance, exact information on this subject is still signally inadequate. One of the greatest obstacles to experimental investigation of the problem has been the lack of an effective method for evaluating drought resistance. The first step in the study discussed here was to devise an apparatus in which a direct test of drought resistance could be made. By means of the direct test, it is possible to study the influences exerted on drought resistance by external and internal factors. This report presents evidence that the tests made provide a real index of the inherent drought resistance of coniferous seedlings, and evidence as to the influence of previous moisture supply on the drought resistance of the principal conifers of the Lake States. It presents data, also, regarding the persistence of the improved drought resistance built up by treatments applied to seedlings in the nursery.

[1] Received for publication December 7, 1938.
[2] Acknowledgment is due several other members of the staff of the Lake States Forest Experiment Station who have contributed materially to the study discussed.
[3] Italic numbers in parentheses refer to Literature Cited, p. 20.

The field tests described in this paper were made at Cass Lake, on the Chippewa National Forest, Minn., with plants grown in nearby forest nurseries.

REVIEW OF LITERATURE

Both direct and indirect methods have been used to study drought resistance. Studies by indirect methods have contributed much to an understanding of the morphological and physiological responses of plants to drought. However, owing to the extreme complexity of the problem, no simple and clear-cut criteria have been developed whereby the drought resistance of a given plant may be determined with accuracy. Investigators have now begun to adopt direct exposure to drought as the method of determining hardiness. Maximov (*10*), in an exhaustive study of factors influencing drought resistance, concluded that the capacity to endure prolonged wilting is one of the most important characters influencing drought resistance in plants. Although this conclusion has not yet been verified by extensive experimental evidence, it is significant that students of the closely related problem of cold resistance, having encountered contradictory results from indirect tests (*3*, *5*, *12*, *17*), now rely almost exclusively upon direct exposure to low temperature to determine winter hardiness.

Marshall (*9*) studied the reactions of several species of conifer seedlings as they wilted, became desiccated, and died after suspension of watering. Tumanov (*18*, *19*) and other Russian investigators, after subjecting plants to soil dryness, have taken as a criterion of drought resistance the percentage survival or the relative resistance to loss of leaf and stem tissue through desiccation. Krasnosselsky-Maximov (*8*) subjected plants to hot, dry air in a wind tunnel in order to investigate reactions to drought.

The relative resistance of varieties of maize to combined drought and heat was investigated by Hunter, Laude, and Brunson (*6*) by use of a closed, heated chamber. As this chamber was operated at 140° F. (60° C.) and at a relative humidity of about 30 percent, the injuries noted may have resulted more from excessive heat than from drought.

For testing the drought resistance of varieties of wheat a more elaborate apparatus was used by Aamodt (*1*). This consisted of a glass-enclosed tunnel through which air, heated to constant temperature, was blown at a velocity of about 6 miles per hour. A revolving chain belt has recently been installed in this apparatus to insure uniform exposure of the plant material.[4] In recent work with this apparatus, which is operated in a greenhouse, Peto [5] has encountered difficulty in comparing the results of replicate tests, owing to lack of control over light intensity.

Tumanov (*18*) and Kondo (*7*) have shown that crop plants subjected to periodic wilting while in the growing stage attained greater resistance to subsequent drought than those grown with an abundant moisture supply. Aamodt and Johnston (*2*) cite several studies confirming this finding and present data of their own indicating that wheat plants can be hardened by subjecting them either to soil dryness or to hot, dry wind. Data presented by Marshall (*9*) indicate that coniferous seedlings grown in a soil deficient in moisture survived drought longer than those grown in a wet soil.

[4] According to Prof. K. W. Neatby, of the University of Alberta.

[5] PETO, HOWARD B. A METHOD OF STUDYING DROUGHT RESISTANCE IN PLANTS. 1937. Unpublished manuscript, Univ. of Alberta.

METHOD EMPLOYED TO TEST DROUGHT RESISTANCE

In preliminary experiments it was found almost impossible to get a reliable test of the relative drought resistance of coniferous seedlings simply by discontinuing the water supply. If the plants remained in the greenhouse, the soil dried unevenly in the different containers. If this difficulty was overcome by adding water, 3 to 6 weeks were required before all plants died. Furthermore light intensity, temperature, relative humidity, and air movement, all of which affect rate of transpiration, were found to be unequal at different locations within the greenhouse. When the same method was applied out of doors, protection from rain was necessary and even then the test required 2 to 4 weeks.

If the influence of various factors on drought resistance is to be studied effectively, not only must all plants in each test lot be uniformly exposed to drought but exposure must be so severe that the results will be free from the influence of secondary hardening. Such hardening was found often to mask the influence of the primary factors in tests of 2 or more weeks' duration. Therefore a procedure was adopted involving continuous exposure to acute desiccation.

DROUGHT MACHINES

The first drought machine used in this study was an improvised apparatus consisting of a large galvanized-iron can inverted over a revolving table on which the potted plants rested. Pans containing calcium chloride were placed under the table, to which fins were attached for fanning air over the desiccant. Tests with this machine, which was constructed in 1932, convinced the senior author of the possibilities of this method. Consequently a carefully planned machine was constructed in 1933. A description of the essential features of this apparatus was published in 1934 (*13*). With slight modifications, the same equipment was used in a study of lethal high temperatures for coniferous seedlings (*15*). While satisfactory in other respects, this machine proved to be too small to hold the quantities of experimental material needed when more than two treatments were involved.

In 1936 a third machine (fig. 1) was built on the same principles as the 1933 model. The table, enlarged to 5 feet in diameter, will support as many as 240 individually potted plants in four outer rows where air velocity is approximately constant at 5.2 miles per hour. Instead of a squirrel-cage blower such as was used in 1933, which functions inefficiently in a closed system, a positive-pressure blower, capable of delivering air against a pressure of 3 pounds per square inch, was installed. This consists of two oppositely rotating impellers, mounted on parallel shafts, inside an oval housing. This blower delivers per minute 48 cubic feet of air, almost one-half the total air volume of the machine when filled with plants. Air from the top of the chamber is drawn into a pipe 1½ inches inside diameter and is passed through the pump and into the air drier. This consists of a rectangular box of sheet copper in which 10 pairs of trays holding as much as 15 pounds of calcium chloride can be inserted. The interior is arranged in such a manner that air passing through the drier must flow across the surface of each pair of trays in turn. The dry air is forced vertically down onto the plants as they pass by the door. This breaks up the air mass

immediately surrounding the plants, which otherwise tends to rotate with them. A fan mounted at the ceiling of the chamber has been found useful in further breaking up local eddies and in insuring uniform air temperature.

The sheet-metal drum is made in two vertical halves reinforced with angle iron. All joints are gasketed with ¼-inch sponge rubber, so that the entire system is approximately airtight. The exterior is covered with one to two layers of asbestos paper, to prevent too rapid loss of heat. The interior is coated with a powdered-aluminum paint, which reflects and diffuses light. Heat is supplied by an electric

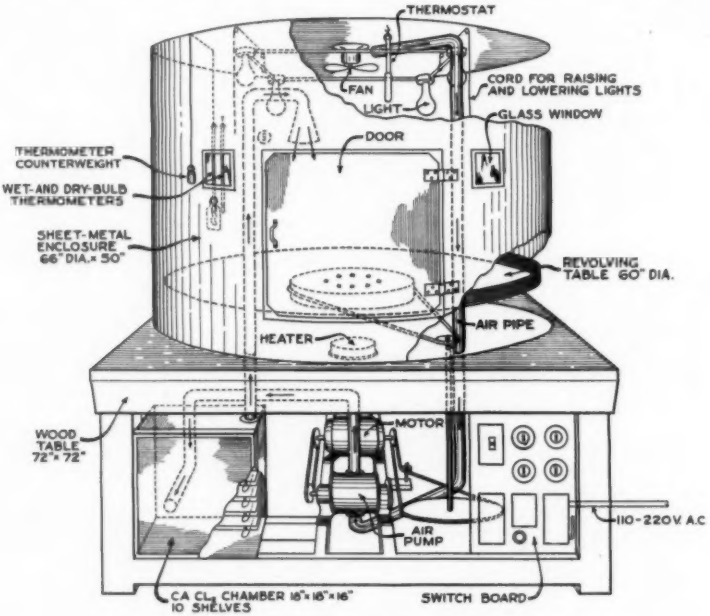

FIGURE 1.—Diagram of 1936 drought machine.

heater mounted under the table and by four lamps totaling 1,000 watts' capacity. These lamps provide, at the level occupied by the plants, a light intensity of 406 foot-candles at a radiation intensity of 0.13 gram-calorie per square centimeter per minute. The heater alone, or the heater and two lamps, can be activated by a mercury thermoregulator sensitive to about 0.1° C. A small low-voltage current through the regulator activates a relay which controls the heater circuit. Both the lamps and the thermoregulator are suspended on springs, which take up vibration. Wet- and dry-bulb thermometers are placed in the stream of outgoing air, which has a current velocity of 443 feet per minute. A special humidifying device (fig. 2) [6] delivers steam into the drought chamber when activated by the humidostat.

[6] This device was designed and constructed by E. E. Aamodt.

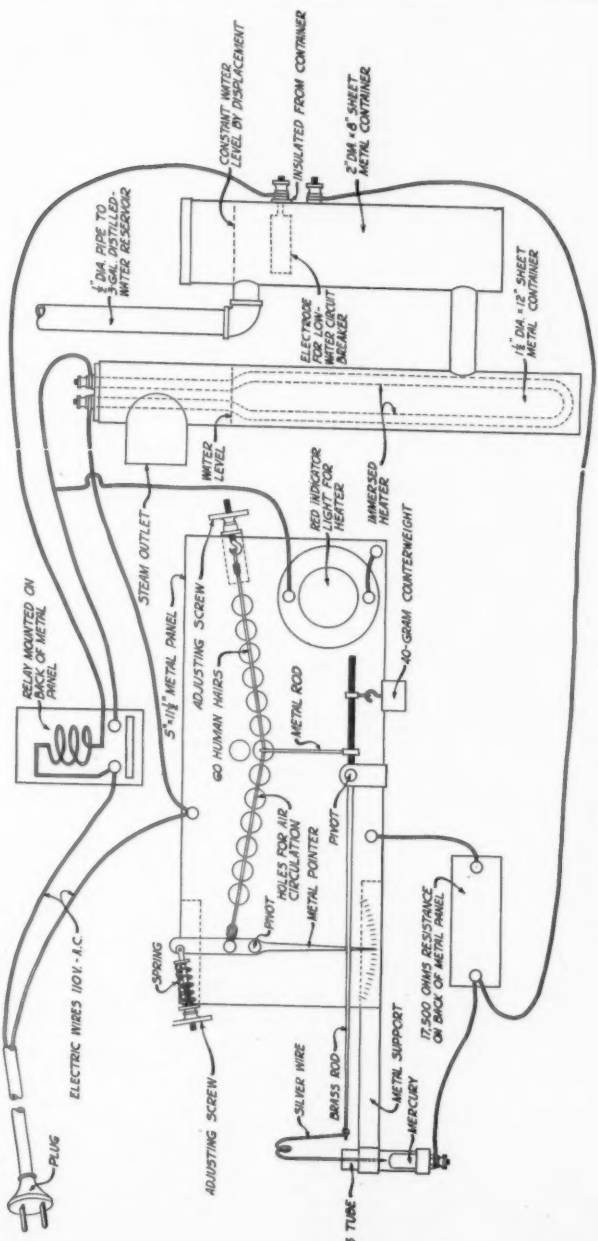

FIGURE 2.—Diagram of humidifier used in 1936 drought machine.

The revolving table is mounted on the front-wheel assembly of a standard motorcar. Even when fully loaded with more than 500 pounds of potted plants the table makes 31 revolutions per minute, with negligible friction. A single ½-horsepower motor drives both the air pump and the table. All speed reduction is by pulley and belt, which allows flexibility in starting and minimizes friction.

Most tests are run at a temperature of 35° to 37° C., which is well below lethal values for conifers (*15*). The atmospheric humidity is held up to 20 percent. The accuracy of control possible in this machine, and also in the 1933 machine, is indicated by the temperatures and humidities for 1937 tests (table 1). Since temperature is controlled solely by the addition of heat, it is necessary that the room in which the machine is used remain sufficiently below the operating temperature to dissipate the heat given off by the lamps. The room in which the 1936 machine is operated is maintained at constant temperature by heating and cooling coils activated by thermostats. It has been found that in this room the humidity within the machine can be controlled more easily by venting a small quantity of air than by circulating the air over a desiccant.

TABLE 1.—*Temperatures and humidities in drought machines during tests conducted in 1937*

1936 machine					1933 machine				
Date of test	Temperature		Relative humidity		Date of test	Temperature		Relative humidity	
	Mean	Standard deviation	Mean	Standard deviation		Mean	Standard deviation	Mean	Standard deviation
	° *C.*	° *C.*	*Percent*	*Percent*		° *C.*	° *C.*	*Percent*	*Percent*
May 4	36.0	0.4	22	5	July 16	37.8	0.5	27	4
May 12	36.2	.6	19	3	July 21	37.8	.5	24	3
June 3	36.1	.2	22	2	July 27	37.9	.3	31	7
July 6	36.1	.5	21	1	Aug. 4	37.9	.7	29	4
July 13	36.5	.9	24	3	Aug. 10	37.5	.8	27	3
Aug. 2	36.1	.6	21	2	Aug. 17	37.2	.3	24	2
Aug. 11	36.0	.2	21	2	Aug. 24	37.6	.3	29	4
Sept. 2	35.9	.5	23	3	Sept. 1	37.7	.4	26	4
Sept. 9	35.7	.4	20	2	Sept. 10	37.8	.3	27	5
Oct. 5	36.2	.6	21	1					
Oct. 14	36.8	.7	21	1					

HANDLING OF PLANT MATERIAL

The species used in the tests reported were red pine (*Pinus resinosa*), northern white pine (*P. strobus*), and jack pine (*P. banksiana*). Stocks of individual species used for comparative tests were in each case derived from the same seed source.

Although other factors could be held essentially uniform, the plant material was likely to vary widely from one test to another. To avoid error in interpretation from this cause, the material used in every individual test included control plants. Experience has shown that 20 plants are sufficient to give a reliable average, hence as many as 12 variations of treatment can be tested simultaneously in the 1936 machine. The fact that coniferous seedlings of the ages used vary appreciably in size made it necessary to arrange the tests in such a way that the effect of size would not obscure the effect of treatment.

This was accomplished by using a factorial design in which size was one of the variables. The data obtained were treated by the analysis-of-variance method (*4*).

When tests were made on seedlings lifted directly from nursery beds, care was taken to select plants within the same size range to represent each treatment. This was done sometimes by ocular comparison, but more often by weighing each plant. Repeated analyses have shown that minor variations in size of seedlings, such as 1 to 2 grams for 2–0 stock, do not appreciably affect relative drought resistance. On the other hand large variations in size cause very important differences in resistance, the resistance increasing with size.

The seedlings were potted individually in No. 2 or No. 3 tin cans or in 4-inch clay pots. Fine sand having a wilting coefficient of approximately 1.0 percent was used as a potting medium. This was thoroughly mixed and brought to 5-percent moisture content before potting. The plants were placed in the drought machine immediately after potting and were allowed to remain without additional water until all the needles or the stem became brittle, determined by touching or breaking. The plants had then a mean moisture content, based on dry weight, of about 10.5 percent with a standard deviation of 3.1 percent. This end point was chosen as the best practicable criterion of death. The length of time each plant remained alive was used as an index of its drought resistance.

VALIDITY OF MACHINE TESTS

In order that the severity of drought to which plants are exposed in the machine may be appreciated more fully, data are presented in table 2 as to weather conditions at Cass Lake, Minn., during the 1936 drought, a period of 58 days in which precipitation totaled only 1.21 inches. During the latter part of that period the soil moisture on the plot where field tests were conducted was but little above the wilting coefficient. To a depth beyond 6 inches, the sandy soil would flow freely between the fingers. On only 8 of the 58 days did the temperature at 5 p. m. approach that maintained in the machine, but 7 of these days were consecutive. The relative humidity reached low values on only 3 days. The wind velocity in the field, on the other hand, appears to have been somewhat greater than that in the machine. It was measured by an anemometer operating 25 feet above the ground level, where wind velocities are from 50 to 100 percent greater than at the level occupied by seedling conifers. Exposure in the machine to a continuous wind of 5.2 miles per hour, therefore, was probably more severe than exposure near the ground level in the field.

TABLE 2.—*Weather conditions at Cass Lake, Minn., from July 1 to Aug. 27, 1936, as shown by readings taken at 5 p. m.*[1]

Range of temperature	Occurrence	Range of relative humidity	Occurrence	Range of wind velocity	Occurrence
° C.	*Days*	*Percent*	*Days*	*Miles per hour*	*Days*
12–20	7	76–100	6	0– 5	15
21–26	18	51– 75	16	6–10	31
27–32	25	26– 50	33	11–16	12
33–39	8	18– 25	3	---------	-------

[1] Data from records in district ranger's office, Cass Lake, Minn.

The results of two tests conducted in 1934 afford evidence of the validity of machine tests. One of these was a test of the value of various foliage sprays in reducing drought mortality (*16*). A total of 49,000 seedlings of red pine, white pine, and white spruce (*Picea glauca*) were sprayed and planted in replicated blocks in the field. The percentage survival of these plants was compared with the relative drought resistance of sprayed seedlings of the same species as revealed by 12 tests made in the machine. The machine tests agreed with the field tests in indicating that one spray was slightly injurious and the others ineffective. During the same summer, preliminary experiments were begun to test the effects of fertilizers on drought resistance. The field and machine tests cannot be considered entirely comparable in this case, owing to great differences in soil volume and in moisture content; however, the results of machine tests were in general agreement with field survival.

Further evidence that the results of machine tests are truly indicative of capacity to resist natural drought was obtained through a series of field and machine tests carried out to determine the relative resistance of stock from two nurseries, the Lydick and the experimental.[7] Most of the plant material used in this study was grown in these nurseries. During the summer of 1936 plantings were made in a dry exposed field on July 30 and August 11, at the height of the dry period, and the results of these plantings were compared with those of tests made between July 23 and September 17 in the 1933 machine. Two types of tests were made, wet and dry. For wet tests in the machine, the soil was saturated before the plants were placed in the machine but no water was added thereafter. The plants remained in the machine until dead. For wet tests in the field, made on August 11, 1 pint of water was poured around each seedling immediately after planting. For dry tests in the machine, seedlings were potted in air-dry soil, placed in the machine for from 4 to 6 hours, watered, and removed to a shaded bed in the nursery; for dry tests in the field, made on July 30, seedlings were planted in dry soil and were left overnight before being watered. All plants were examined daily to determine the number dead. The greatest differences in survival between the plants from the two nurseries, respectively, for dry tests in the machine and for both kinds of field tests, occurred between the second and the third week. The number surviving at this time was used as an index of relative resistance.

The results from tests of drought resistance by the two methods are shown side by side in table 3. Survival percentages for field tests are based on 25 plants, those for machine tests on from 10 to 20 plants. The results from all tests on red pine are in agreement, though that of one field test failed to show significance. Neither the machine-test results for white pine nor those for jack pine are in agreement. Either the differences between the plants from the two nurseries were slight or—what is more likely—they fluctuated during the season. Results of field tests on jack pine also were in disagreement. Both methods of testing gave results indicating that no consistent differences prevailed between the two lots of jack pine.

An attempt was made to repeat these tests during 1937. A test in early July gave a valid and consistent comparison, shown in table 3.

[7] A description of these nurseries is given on p. 11.

A second test made in early August also gave a consistent comparison between machine and field survival; however, in this test the plants in the field received only a mild exposure to drought. (The 1937 reversal in relative drought resistance of stock from the two nurseries is explained in a later section.)

It may be stated that so far as evidence is available there is substantial agreement between results of machine tests of drought resistance and field survival during dry periods.

TABLE 3.—*Comparison of results of machine tests with field survival of 2-year-old stock* [1]

Year and species	Soil condition at start of test	Survival in machine tests			Survival in field tests		
		Lydick stock	Experimental stock	Significance of difference [2]	Lydick stock	Experimental stock	Significance of difference [2]
1936		*Percent*	*Percent*		*Percent*	*Percent*	
Red pine	Dry	20	60	0.04	52	68	0.30
		Days	*Days*				
	Wet	4.7	5.5	.02			
		5.2	6.1	.01	28	68	.01
		5.6	7.3	.01			
Northern white pine	do	5.3	4.6	.01	8	56	.01
		6.6	6.9	.30			
		Percent	*Percent*				
	Dry	20	70	.01	36	64	.05
Jack pine	do [3]	27	60	.06	80	56	.07
		Days	*Days*				
	Wet [3]	5.3	4.7	.07	52	68	.30
1937							
Red pine	Moisture content 5 percent.	5.0	4.2	.01	100	30	.01
		6.7	4.3	.01	94	86	.01

[1] 2-0 stock except as noted; i. e., 2-year seedlings.
[2] Determined by Fisher's "*t*" test (*f*) for machine tests in wet soil, by chi-square test for all others. Only values of 0.05 or less are considered to indicate significance.
[3] 1–1 stock; i. e., 1-year seedlings transplanted for 1 year before field planting.

IMPROVING DROUGHT RESISTANCE BY REGULATING MOISTURE SUPPLY

GREENHOUSE EXPERIMENTS

On April 24, 1936, 22 plants each of 3-year-old red pine, 3-year-old northern white pine, and 2-year-old jack pine were potted in 4-inch clay pots each containing approximately 1,000 grams of soil. The plants were watered daily during the next 10 days. Then the plants of each species were divided into two groups representing the same range of size and vigor. Thereafter one group was watered daily or twice daily to maintain the soil moisture between 18 and 22 percent. The second group was watered every third or, in extremely warm weather, every second day; this allowed the soil moisture to vary between 5 and 20 percent. The wilting coefficient of the greenhouse soil used was 3.4 percent. This treatment continued for 71 days. The pots were then brought to equal moisture content and placed in the 1936 drought machine, where each plant received daily two applications of 50 grams of water. The temperature in the machine was held constant at 44° C.; the relative humidity averaged 27.5 percent. For all three species, drought resistance was significantly higher in the plants that had been subjected to periodic soil dryness (table 4).

TABLE 4.—*Influence of exposure to periodic soil dryness on drought resistance of pine seedlings*

Date of test	Species and age class [1]	Total plants used	Length of treatment	Average survival in machine	
				Stock watered daily	Stock watered every third day
		Number	*Days*	*Days*	*Days*
July 14, 1936	Red pine (2–1)	22	71	4.5	[2] 6.4
	Northern white pine (2–1)	22	71	3.6	[2] 4.6
	Jack pine (2–0)	22	71	5.2	[2] 7.3
June 21, 1937	do	120	30	5.0	5.2
July 20, 1937	do	80	60	4.5	[2] 4.9
Jan. 10, 1938	Jack pine (1–0) [4]	50	43	3.8	[2] 4.4
	do.[5]	50	43	3.0	[2] 3.5

[1] In the age-class symbol, the first figure indicates years in seed bed; second figure, subsequent years in transplant bed.
[2] Values significantly greater at 0.01 level for periodic than for daily watering.
[3] Values significantly greater at 0.05 level.
[4] Soil not replaced.
[5] Soil replaced.

The experiment was repeated in 1937 with 2–0 jack pine. Potted seedlings were watered uniformly for 60 days; they were then given differential watering like that in the preceding experiment, one lot for 30 days and one lot for 60 days. At the end of each period of treatment seedlings were transplanted from the clay pots into cans containing sand with a 5-percent moisture content, and placed in the 1936 machine. No water was added thereafter. The machine was operated at a temperature of 37° C. and a relative humidity of 20 percent. The seedlings that had been watered infrequently for 30 days showed a statistically insignificant advantage in resistance; for those that had been treated for 60 days, the difference was significant at the 0.05 level (table 4).

In a third experiment, 1-year-old jack pines were transplanted to 4-inch clay pots on September 15, 1937. These were watered daily until November 29; then two uniform lots of 50 plants each were selected for treatment. The lot that was to be watered infrequently was placed on a bare wooden table, to facilitate evaporation from the soil and pots; its soil moisture was allowed to fluctuate between 5 and 20 percent. The lot to be watered daily was placed on a greenhouse bench filled with moist sand; its soil moisture was maintained between 18 and 22 percent.

After 43 days of this treatment 25 plants of each lot were removed from the clay pots, shaken free of soil, and repotted in tin cans containing sand with a 5-percent moisture content. The pots containing the remaining plants were saturated with moisture and allowed to drain 1 hour; then each of the plants, with soil intact, was transferred to a tin can. No water was added during the test, which was made in the 1936 machine, operated at a temperature of 41.2° C. and a relative humidity of 10.8 percent. Treatment of the data by the analysis-of-variance method indicated that the plants infrequently watered had a highly significant advantage in drought resistance (table 4).

In addition to these experiments 19 other tests, involving a total of 988 plants, have been carried out to determine the effect of watering methods on drought resistance of coniferous seedlings grown in the greenhouse. The results, although not always statistically significant, have been in general agreement with those of the tests described.

NURSERY EXPERIMENTS

The practical value of controlled watering as an expedient for increasing drought resistance of coniferous seedlings was demonstrated in 1936 and in 1937 by large-scale experiments in two nurseries.

To understand clearly the significance of the several nursery tests, a few important differences between the two nurseries must be borne in mind. The Lydick, a 66-acre Forest Service nursery at Cass Lake, Minn., prior to 1937 had been producing stock less desirable in quality than that grown in the experimental nursery, located 5 miles to the southeast. The difference was ascribed to a variety of causes, of which the better soil in the experimental nursery and the method of watering used there were considered to be the more important. The soil in the Lydick nursery is a fine sand which, during 1936 and 1937, was greatly improved by heavy applications of compost. From the establishment of this nursery in 1933 until the spring of 1937 it was the nurserymen's practice to apply water liberally whenever the beds appeared dry. During the hot, dry period of 1936, in order to minimize losses the beds were watered daily. In 1937, artificial watering was reduced in an effort to produce more resistant stock.

The experimental nursery is located in a 3½-acre clearing surrounded by a 50-year-old aspen stand. Owing to the protection afforded by the timber and to the higher moisture-holding capacity of the fine sandy loam soil, this nursery does not require such frequent watering as the Lydick. As the purpose of establishing this nursery included simplification of care together with production of hardy planting stock, artificial watering has been held to a minimum. Normally, water is applied only during the germination period. In 1936, however, it was necessary to water during July and August, applying a total of 1.62 inches of water to first-year seedbeds and 0.32 inch to 2- and 3-year beds.

For reasons not fully understood, the jack pine stock from the experimental nursery hardens later in the fall than that from the Lydick. Consequently, if planted in the fall it suffers from winter injury.

TABLE 5.—*Field survival in 1936, by species and age class and by nursery of origin, of pine seedlings from the Lydick and experimental nurseries planted in the fall of 1935*

Date examined	2-0 jack pine		1-0 jack pine		2-0 red pine	
	Lydick	Experimental	Lydick	Experimental	Lydick	Experimental
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
June 24	70	65	85	79	80	79
July 30	49	54	60	65	47	58
Aug. 11	37	47	50	54	30	46
Sept. 8	30	41	39	45	----------	40

The behavior of stock from the two nurseries when planted in the fall is illustrated by the field survival in a special test planting [8] established in 1935, shown in table 5. The differences in survival of

[8] Planned and executed by R. H. Blythe, Jr., and Paul Zehngraff.

red pine during the summer of 1936 are statistically significant. Jack pine stock from the Lydick nursery showed significantly greater losses during the summer than that from the experimental nursery. The Lydick stock had a higher survival over winter, however, and the difference in survival at the end of the 1936 season is not significant.

In addition to this experiment, about 60 acres were planted in the fall of 1935 with jack pine stock from the two nurseries. No attempt was made to lay out special comparative plots, since the planting covered irregular areas in the east tier of forties of a section. A count of survival made in August 1936 gave results shown in table 6. Stock from the experimental nursery showed a distinct advantage over that from the Lydick.

To check the reliability of the evidence that stock produced in the experimental nursery was better suited for field planting than that produced in the Lydick, the series of field and machine tests mentioned in an earlier section were carried out during 1936 and 1937. The quantity of water applied during the 30-day period preceding machine tests of red pine, and results with this stock, are shown in table 7. In each of the three tests made in 1936, stock from the experimental nursery proved to be more drought resistant than that from the Lydick. The quantity of water supplied to the Lydick stock preceding the first two tests was approximately three times the quantity supplied to the experimental stock. Artificial watering was discontinued in both nurseries during September, but increased drought resistance of experimental stock induced by watering methods used during July and August persisted.

TABLE 6.—*Field survival on Aug. 21, 1936, of 2-year-old jack pines from the Lydick and experimental nurseries planted the previous fall*

Forty No.	Lydick nursery stock		Experimental nursery stock	
	Age class	Survival	Age class	Survival
	Years	*Percent*	*Years*	*Percent*
32	2-0	28	1-1	55
31	2-0	36	1-1	59
22	2-0	35	2-0	58
21	2-0	19	2-0	51

The tests on seedling stock in 1937 showed Lydick stock to have greater resistance than experimental stock—a direct reversal of the 1936 results. However, the data for 1937 show relatively little difference between the two nurseries as to water supply. Presumably, because of the low moisture-holding capacity of its soil, less moisture was available in the second-year seedbeds of the Lydick nursery than in those of the experimental nursery, even though the former received artificial watering. Unfortunately no record of soil moisture is available. It is significant, however, that the Lydick nurseryman was making a deliberate effort to increase the hardiness of his stock by limiting artificial watering. The fact that he was obliged to apply water whereas no artificial watering was needed in the experimental nursery is evidence of drier soil conditions in the Lydick. Furthermore, each time seedling stock was lifted for testing it was noticed that the Lydick beds were drier than the experimental beds.

TABLE 7.—*Drought resistance of red pine in 1936–37, by nursery of origin and by previous watering*

Class of stock and date of test	Total plants used	Watering[1] during 30 days preceding test				Average survival in machine		Significance of difference
		Days water was applied		Total quantity of water applied				
		Lydick	Experimental	Lydick	Experimental	Lydick	Experimental	
1936								
2-year seedlings:	*Number*	*Number*	*Number*	*Inches*	*Inches*	*Days*	*Days*	
July 23	20	24	6	4.12	1.29	4.7	5.5	0.02
July 29	20	26	7	4.15	1.29	5.2	6.1	.01
Sept. 30	30	13	12	2.26	2.21	5.6	7.3	.01
1937								
July 15	40	16	9	6.92	5.33	5.0	4.2	.01
July 21	40	17	11	7.42	6.06	4.7	4.1	.01
July 27	40	17	10	8.32	6.85	6.3	4.6	.01
Aug. 16	40	11	7	2.53	1.81	6.7	4.3	.01
Sept. 9	40	11	6	6.22	5.50	8.2	5.2	.01
1937								
2-1 transplants:								
July 2	32	13	9	4.71	1.73	3.6	6.0	.01
July 2	32	13	9	4.71	1.73	3.6	5.2	.01
Aug. 1	32	19	9	12.58	6.87	5.3	6.4	.01
Sept. 2	32	10	8	5.74	5.36	4.1	5.8	.01

[1] Includes rainfall and artificial watering. Experimental nursery stock received only 0.32 inch of artificial watering in 1936 and no artificial watering in 1937.

The Lydick transplants tested in 1937 had been set out adjacent to first-year beds, where they received more frequent and abundant watering than the 2-year-old seedlings. These transplants proved far less drought resistant than similar stock from the experimental nursery which received no artificial watering.

The reversal in comparative drought resistance between seedlings and transplants is directly related to quantity of water received. Seedlings deliberately hardened in the Lydick nursery were more resistant than experimental seedlings, but transplants abundantly watered in the Lydick nursery were less resistant than experimental transplants. This is concrete evidence that drought resistance of coniferous seedlings can be improved by controlling the moisture supply in the nursery.

Three tests involving differential treatments applied within a single nursery may be cited to strengthen this conclusion. During 1936 one portion of a bed of 2-year-old red pine in the experimental nursery was allowed to go through the summer without artificial watering; the remainder of the bed received 0.32 inch of water, in seven applications. On the first of October, plants from both portions of the bed were tested in the 1933 drought machine. The plants from the watered portion of the bed survived an average of 6.2 days; those from the unwatered portion, 6.9 days. Since the probability that this difference was due to chance is less than 0.01, it may be considered highly significant.

In another experiment, 1-year-old jack pines were transplanted to plots in the Lydick nursery in the spring of 1935. All plots received

normal watering from the overhead sprinkling system. Half the plots received, in addition, a daily application of 0.4 inch from sprink-

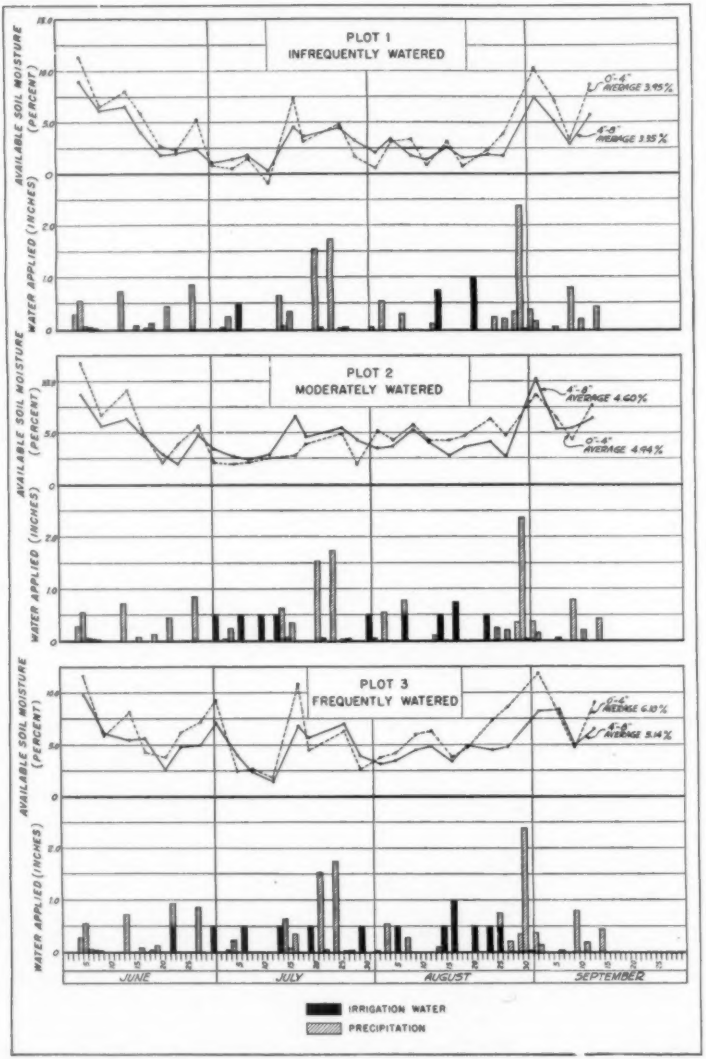

FIGURE 3.—Variations in soil moisture of three jack pine plots in the Hayward, Wis., nursery, during the growing season of 1937.

ling cans. After being thus treated for two growing seasons, the plants were tested in the 1933 drought machine. Plants from plots watered daily survived 4.8 days; plants from plots watered normally, 5.9

days. Though only 10 plants representing each treatment were tested, the difference was significant at the 0.02 level.

A third experiment,[9] also involving jack pine, was conducted at the Forest Service nursery at Hayward, Wis., during the summer of 1937. In this case the moisture content of the soil was determined at biweekly intervals. The soil in this nursery was a medium sand having a wilting coefficient of 3.5 percent. Water was applied to three plots with a sprinkling can. The quantity of water applied, and the moisture available in the soil throughout the growing season, are shown graphically in figure 3. The soil moisture of the three plots showed similar seasonal trends. At critical periods the soil of the infrequently watered plot was always the driest. Of the 27 soil samples from this plot, 13 had less than 2.5 percent available moisture. A test of 26 seedlings from each of the three plots was made in the 1936 drought machine on October 25, the results of which are given in table 8. The infrequently watered plants were again significantly more resistant than those receiving a more abundant moisture supply.

TABLE 8.—*Effect of watering treatments in the Hayward, Wis., nursery in 1937 on drought resistance of 2–0 jack pine*

Watering treatment	Average available soil moisture at 0- to 8-inch level	Survival in machine
	Percent	*Days*
Infrequent	3.6	[1] 7.9
Moderate	4.8	6.6
Frequent	5.6	6.7

[1] Significantly greater at 0.01 level.

PERMANENCE OF INDUCED DROUGHT RESISTANCE

Obviously, building up drought resistance in nursery stock by controlled watering would result in no practical gain unless the improved resistance persisted for at least one season after treatment.

Tests in 1936 having shown that controlled watering in the experimental nursery had induced greater drought resistance in 2-year-old red pine than that occurring in similar Lydick stock, experiments were carried out in 1937 on plants from the same beds to discover whether this difference was still retained, independent of the growing conditions prevailing during that year. On May 14, 2- and 1-year-old seedlings grown in both nurseries were transplanted in randomized rows in four replicate beds in each nursery. The beds in the Lydick nursery were located adjacent to first-year seedbeds, where they received during the growing season a total of 7.7 inches of water from an overhead sprinkling system; the beds in the experimental nursery received no artificial watering. Samples from each bed were lifted at monthly intervals and brought to St. Paul for testing in the 1936 drought machine. At St. Paul each consignment was divided into halves, which were tested separately, the second being stored at 5° C. until the test of the first was completed. A temperature of 36° C. was maintained throughout these tests, and a relative humidity of from 19 to 25 percent. The

[9] Planned and executed by J. H. Stoeckeler and J. W. Jay, Lake States Forest Experiment Station.

results from each test were analyzed by the variance method to determine the significance of differences. Averages of survival in the machine are shown in table 9.

TABLE 9.—*Drought resistance of red pine nursery stock transplanted* [1] *in May 1937, in days of survival in drought machine*

Date lifted, 1937	Date machine test began, 1937	Plants per treatment	Stock lifted in third year from—				Stock lifted in second year from—			
			Lydick nursery, first two years in—		Experimental nursery, first two years in—		Lydick nursery, first year in—		Experimental nursery, first year in—	
			Lydick	Experimental	Lydick	Experimental	Lydick	Experimental	Lydick	Experimental
		Number	*Days*	*Days*	*Days*	*Days*	*Days*	*Days*	*Days*	*Days*
May 1	May 4	30	6.18	.....	.....	2.97	3.66	.....	.....	3.20
May 1	May 12	30	5.32	.....	.....	2.99	3.94	.....	.....	2.79
June 1	June 3	25	3.48	.....	.....	4.51	4.47	.....	.....	3.86
July 1	July 6	28	3.61	4.45	4.06	6.04	2.87	2.74	2.93	3.06
July 1	July 13	28	3.65	4.61	3.76	5.16	3.06	2.70	2.52	2.69
July 30	Aug. 1	28	5.27	5.58	5.70	6.38	4.95	4.98	4.82	5.14
July 30	Aug. 11	28	4.46	4.88	4.76	5.72	3.92	4.59	4.36	4.57
Sept. 1	Sept. 2	28	4.13	4.69	5.57	5.82	4.08	4.31	4.17	4.58
Oct. 1	Oct. 5	28	5.69	7.02	5.79	6.12	5.55	5.00	5.16	4.37
Oct. 1	Oct. 14	28	6.09	6.70	5.88	6.45	5.47	5.32	5.84	5.27

[1] With the exception of plants used in May and June tests, which were lifted directly from seedbeds.

Of the seedlings lifted on May 1, those from the Lydick nursery were more resistant. As this was at the beginning of vegetative activity, results with this lot are of interest only as indicating seasonal variation. By June 1, stock from the experimental nursery was more resistant.

The first tests of transplanted stock were made on July 6. The 3-year-old stock from the experimental nursery, which by actual test was known to have been more drought-resistant than Lydick stock in 1936 (table 7), was significantly more resistant in 1937 than Lydick stock treated in the same way during the third year. The second-year stock not tested in 1936 showed insignificant differences in 1937.

This experiment clearly indicates that increased drought resistance brought about in 2-year-old red pine seedlings by control of water supply in the nursery persists during the third year. Whether treatments applied to seedlings during their first year can affect significantly their drought resistance during the second year remains an open question. Inasmuch as the ratio of the quantity of tissue developed during the first year to that developed during the second year is much less than the ratio of second-year tissue to third-year tissue, it is not surprising that no clear-cut evidence of a carry-over of resistance by 1-year-old seedlings was detected.

The advantage of the experimental nursery watering regime over that maintained in the Lydick nursery is further demonstrated by this experiment. Stock that had been transplanted at the beginning of the third year from the Lydick nursery to the experimental nursery (table 9, column 6 stock) showed significantly greater drought resistance, in six out of seven tests, than similar stock transplanted within the Lydick nursery (column 4). Conversely, experimental stock transplanted to the Lydick nursery (column 5) showed significantly

less drought resistance, in five out of seven tests, than similar stock transplanted within the experimental nursery (column 7). Also, Lydick stock transplanted at the beginning of the second year to the experimental nursery (column 10) became more resistant than similar stock transplanted within the Lydick nursery (column 8).

SUPPLEMENTAL FINDINGS

EFFECT OF OVEREXPOSURE TO SOIL DRYNESS

Although the drought resistance of conifers can be increased by exposure to soil dryness, caution is necessary to avoid injuring the plants by this treatment. An example of the effect of overexposure is afforded by two experiments made in the spring of 1937. Jack pine seedlings 1 year old were transplanted to dry sand in the late afternoon and allowed to stand overnight. The following morning the soil was saturated with moisture. In the first experiment the seedlings were given one, two, three, four, and five exposures to dry sand on consecutive days. Half of each lot of seedlings was tested the next day after all these treatments were completed. Drought resistance decreased as number of exposures increased (table 10). The remaining half was tested after being given 24 days of normal watering in which to recover. All except those that had been given five exposures survived 6 days in the machine, which presumably represents the equivalent of their resistance before treatment. In the second experiment, the plants were left overnight in air-dry sand, than watered and allowed 16, 8, 4, and 1 day to recover before being tested. Control plants were transplanted to moist soil. Differences in survival resulting from differences in time allowed for recovery were not significant, nor were differences in survival among the control lots; but the plants transplanted to dry sand showed significantly less resistance than the control plants.

TABLE 10.—*Decrease in drought resistance of 1-year-old jack pine seedlings resulting from exposure of roots to dry sand* [1] *at night*

Nights of exposure	Days of recovery	Plants treated	Survival in machine	
			Initial test [2]	Test 24 days later [3]
Number	*Number*	*Number*	*Days*	*Days*
1	4	32	4.6	6.1
2	3	32	3.9	6.4
3	2	32	3.7	6.3
4	1	32	3.3	6.1
5	0	32	3.1	4.8
0	----------	32	5.9	6.2

[1] The plants not exposed to dry sand (for which data are given in last line) were drawn from the same lot of dormant seedlings but were tested 38 days later than the treated plants.
[2] Differences greater than 0.74 are significant at the 0.05 level, according to Fisher's (4) analysis-of-variance method.
[3] Differences greater than 1.1 are significant at the 0.05 level.

In 1934 1-year-old jack pine and 2-year-old red pine seedlings were transplanted to a dry, sandy field, where they remained 2 months. By the end of that time more than half of them had died, and the remainder had been stunted in growth. The drought resistance of the more vigorous survivors was compared with that of fresh stock

from the nursery. The seedlings from the field proved to be markedly less resistant than the nursery stock.

It is concluded that after severe exposure the plants do not regain their original resistance unless allowed a rather prolonged period of recovery.

ROOT DEVELOPMENT AND DROUGHT RESISTANCE

The objectives of the experiments did not include determination of the influence of root development on drought resistance; however, some observations made on this point are worthy of consideration. Plants subjected to moderate drought tended to develop relatively more extensive root systems and smaller tops than those grown in soil continuously moist. In general, plants with well-developed roots were more resistant to drought than those with less extensive root systems. Yet this was not always the case; among the red pines tested in 1937, the more resistant were not always those with either the better-developed roots or the greater root-top ratios (table 11). These facts are presented not for the purpose of belittling the advantages of a good root system during periods of drought but rather to indicate that drought resistance in young conifers does not depend solely upon size of root system or relative root development.

TABLE 11.—*Drought resistance and root development of red pine transplants from both nurseries*

Nursery of origin and date of test, in 1937	Dry weight of roots		Root-top ratio		Survival in machine	
	Lydick	Experimental	Lydick	Experimental	Lydick	Experimental
Lydick:	*Grams*	*Grams*			*Days*	*Days*
Aug. 1	0.25	0.20	0.28	0.30	4.85	5.23
Sept. 1	.57	.50	.21	.17	4.13	5.57
Oct. 1	.60	.90	.32	.46	5.89	5.83
Experimental:						
Aug. 1	.76	.71	.36	.28	5.23	6.05
Sept. 1	1.12	.77	.30	.22	4.69	5.82
Oct. 1	.89	2.10	.34	.61	6.86	6.28

RELATIVE RESISTANCE OF DIFFERENT SPECIES

The experiments reported were not designed to test difference among species. This probably varies a great deal, according to the treatment received by the plants previous to the survival test. However, from the data in table 4 it is seen that jack pine is the most resistant, followed by red pine and white pine. This applies only to stock of about the same size and in the same stage of vegetative development. Further discussion of this question is deferred until more easily interpreted data are available.

DORMANCY AND DROUGHT RESISTANCE

Resistance to drought varies widely depending upon vegetative activity. This is clearly evident from the survival of plants from the same beds tested at several different times during the course of a season (table 9). Before the beginning of active growth in the spring, drought resistance was relatively high. With the beginning of stem

elongation, drought resistance showed a pronounced decrease, which persisted until active growth ceased in early summer. Thereafter, drought resistance increased.

These tests, together with others not reported in this paper, indicate that the drought resistance of actively growing conifers differs widely from that attained after a short period of hardening. Because of this difference, results of tests made on actively growing stock are likely to be wholly inconsistent with results of tests on similar stock after vegetative activity has declined. It has been found that drought resistance can be most effectively tested after rapid height growth has ceased, during the period in which the newly formed tissue is maturing but before the plant becomes dormant. This is the season at which droughts are most likely to occur.

DISCUSSION

The fact that the relative survival of coniferous seedlings in the drought chamber was in substantial agreement with that in the field during periods of drought clearly justifies the use of artificially produced drought in studies of the drought resistance of conifers. The advantages of machine tests over field survival tests are many: (1) Tests in the machine are free from biotic influences which often disturb tests made in the field. (2) The machine is available for tests at any time, whereas field tests can be made only during dry periods. (3) The close control over environmental factors possible in the machine greatly reduces variability, with consequent improvement in the reliability of results. (4) Tests in the machine are of relatively short duration, hence are admirably suited for study of the fundamental nature of drought resistance. (5) Direct tests in the machine can be duplicated by workers in other regions, and this makes possible a concerted attack upon the intricate problem of drought resistance in plants.

It is concluded from the experiments described that drought resistance of Lake States pine seedlings can be improved by controlling the moisture supply in the nursery, that such control is a simple and practical expedient available in all large-scale nurseries, and that drought resistance induced in 2-year-old conifers by controlled watering persists to an appreciable extent during the third year. It should be emphasized, however, that drought resistance of young conifers depends upon a number of factors, of which moisture supply is only one. Drought resistance is known to be in part associated with region of seed origin (*11*), and preliminary experiments have indicated that it is influenced to an important degree by both light and soil fertility. Doubtless other factors that modify plant structure or nutrition will ultimately be found to affect the intricate internal balance which determines a plant's resistance to drought. Before comprehensive recommendations can be made to the practicing nurseryman, the action and interaction of the factors mentioned must be more thoroughly understood. More must be known, also, of the rate at which changes in drought resistance take place within the plant. Of this important subject little is known at present except that such changes take place rapidly in actively growing plants and very slowly in dormant plants.

SUMMARY

In order to test the drought resistance of potted coniferous seedlings, an improved "drought machine" in which artificial drought can be produced at will was devised. This consists of a thermostatically controlled, illuminated plant chamber with apparatus for drying air over calcium chloride and forcing it through the chamber. The relative drought resistance of different lots of coniferous seedlings as revealed by tests in this machine was found to correspond substantially with the relative field survival of similar lots during periods of drought.

The drought resistance of seedlings of Lake States pines, namely, red pine (*Pinus resinosa*), northern white pine (*P. strobus*), and jack pine (*P. banksiana*), was increased by subjecting the seedlings to moderate soil drought during the period of vegetative activity. This treatment tended to result in greater development of roots in proportion to top, and in smaller size of tops; but improved resistance did not depend solely upon size of root system or upon ratio between roots and top. Resistance built up in red pine through controlled watering during the second year in the nursery persisted to a significant degree during the following season.

Exposure to severe dryness of soil temporarily weakened the plants and, unless followed by an ample period of recovery, rendered them more susceptible to drought.

Improvement of drought resistance of Lake States pine seedlings by controlling the moisture supply in the nursery was demonstrated to be a practical procedure which can be applied in large forest nurseries.

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INHERITANCE STUDIES IN THE INTERSPECIFIC CROSS SOLANUM DEMISSUM LINDL. × S. TUBEROSUM L.[1]

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INTRODUCTION

Hybrids between *Solanum demissum* Lindl. and *S. tuberosum* L. have been used for the purpose of securing commercially valuable varieties immune to late blight and having some tolerance to frost. The two species differ in chromosome number. *S. demissum* has been reported to have 36 haploid and 72 diploid chromosomes, with normal meiotic divisions (*12, 21, 22, 30*).[3] Varieties of *S. tuberosum* have been reported to have 24 haploid and 48 diploid chromosomes, with various abnormalities in the meiotic divisions (*1; 5; 6; 7; 8; 11; 12; 13; 14; 23, p. 113; 24; 30; 31; 33*). This difference in chromosome number and the reversion of the hybrids to the *S. demissum* parent reported by Reddick (*17, 18*) suggested the need for a study of the cytology and the breeding behavior of the hybrids.

THE PARENT FAMILIES

The parents of the interspecific cross on which this work is based were a true breeding form of *Solanum demissum* form *vitlense* Buk., seed of which was originally obtained from Dr. S. M. Bukassov from the Union of Soviet Socialist Republics in 1931, and *S. tuberosum* selection Minnesota 4–39–2–2, a free-flowering, pollen-fertile, fourth-generation inbred seedling of U. S. D. A. 14329, which was a cross of Keeper × Silverskin. The cross was made in 1932 by using *S. demissum* as the pistillate parent.

Thirty-one seedlings of each parent were grown in 1936 for comparison with each other and with their hybrid progeny. The term "parents," as used throughout this paper, refers to these selfed seedling progenies, not to the clones. The parents differed from each other in many characters, a few of which were measured, the means with their standard errors being compared in table 1.

The cotyledons of *Solanum demissum* were much narrower than those of *S. tuberosum*, but no shorter, and the width-over-length or shape index of *S. demissum* was, therefore, much smaller. Terminal leaflets of *S. demissum* also were narrower in proportion to length than those of *S. tuberosum*, though not as markedly so as the cotyledons.

[1] Joint contribution of the Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and Division of Horticulture, Agricultural Experiment Station, University of Minnesota. Paper No. 1592 of the Scientific Journal Series of the Minnesota Agricultural Experiment Station. Part of a thesis submitted to the faculty of the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the degree of doctor of philosophy. Completion of this project was made possible by workers supplied on project No. 4841, Minnesota Works Progress Administration. Received for publication October 17, 1938.

[2] The writer is indebted to Dr. F. A. Krantz, of the Minnesota Agricultural Experiment Station, for supplying the material and for suggestions in connection with this investigation.

[3] Italic numbers in parentheses refer to Literature Cited, p. 37.

They were much smaller in *S. demissum*. Mature leaves of *S. demissum* were only about one-fourth as large in area as the mature leaves of *S. tuberosum*. Stems of *S. demissum* were about one-third as long as those of *S. tuberosum;* internodes were less than one-fourth as long; and stem diameters were one-half as large. Stems of *S. demissum* were all prostrate, while of the plants of *S. tuberosum* seven were classed as erect, five as semierect, and nine as semiprostrate. *S. demissum* is reported to have stolons as much as a yard long (*20*), but at University Farm the few tubers that were set were on stolons approximately 30 cm. long, while stolons of *S. tuberosum* were 5 cm. long. *S. demissum* does not set tubers at University Farm until late in the fall and had set practically no crop by November 1, while *S. tuberosum* had set a fair crop of medium-sized tubers by October 1.

TABLE 1.—*A comparison of progenies from selfed seed of Solanum demissum and S. tuberosum selection 4–39–2–2*

Character		*S. demissum*		*S. tuberosum* 4–39–2–2	
		Number of plants	Mean valve for character [1]	Number of plants	Mean valve for character [1]
Morphological characters:					
Cotyledon width	millimeters	31	3.48±0.11	31	6.00±0.25
Cotyledon length	do	31	8.06± .33	31	7.90± .33
Cotyledon shape index		30	.44± .02	29	.75± .03
Terminal-leaflet width	millimeters	27	10.96± .60	20	25.40±1.46
Terminal-leaflet length	do	27	17.70±1.10	20	32.75±1.80
Terminal-leaflet shape index		27	.63± .01	20	.78± .02
Mature-leaf area	square centimeters	26	6.40± .52	20	28.50±3.96
Mature-stem height	centimeters	27	16.41± .94	21	47.50±2.54
Stem diameter	millimeters	26	3.93± .12	20	7.53± .34
Internode length	do	27	6.31± .38	21	28.09±1.22
Tubers	number	18	7.20±2.65	24	24.54±4.58
Weight of largest tuber	grams	26	1.30±	24	10.70±2.58
Stolon length	centimeters	.....	30.00±	23	4.97± .94
Physiological characters:					
Heat and drought injury [2]		31	1.87± .12	28	1.04± .22
Immaturity [2]		26	5.00± .00	22	.27± .19
Frost injury [3]		26	.00± .00	2	5.00± .00

[1] Standard error is used rather than probable error.
[2] Arbitrary scale, 0–3.
[3] Arbitrary scale, 0–5.

Heat and drought affected *Solanum demissum* more than *S. tuberosum*, all plants of *S. demissum* being injured by the severe heat and drought of July 1936, while 12 of the 28 plants of *S. tuberosum* escaped injury. Plants of *S. tuberosum* matured in early September, while all plants of *S. demissum* were still green during October. Plants of *S. demissum* were uninjured by the first frost of the year on October 1, which killed the two plants of *S. tuberosum* selection 4–39–2–2 immature enough to show frost injury, and all plants of another *S. tuberosum* family growing in the same plot, as well as pepper and eggplants in adjoining plots. *S. demissum* is reported to withstand temperatures of about 27° F. (*2, 3, 4, 9, 16, 17, 27, 32*) and to be late-blight-immune (*3, 4, 10, 14, 17, 18, 19, 20, 25, 26, 27, 28, 29*).

THE F_1 FAMILY

The 15 plants making up the F_1 family were started in the greenhouse in the early spring of 1933 and grown there for 4 months before being moved to the field. They were alike in general appearance and

were intermediate in type between the two parents. Hybrid vigor was especially noticeable in the large leaves and the large showy purple flowers.

Several F_1 hybrids of *Solanum demissum* by *S. tuberosum*, similar in their general habit of growth and characters to the F_1 described above, were grown in 1936. The *S. tuberosum* parents of these F_1 plants were not related to *S. tuberosum* selection 4–39–2–2, the parent of the F_2 and backcross plants used in this study. Characters of these F_1 plants are shown in tables 8 to 20 for comparison with characters of plants of the F_2 families.

Chromosome counts made from root tips of some of the F_1 plants of *Solanum demissum* × *S. tuberosum* selection 4–39–2–2 showed 60 chromosomes, as would be expected from the union of 36 in the ovule of *S. demissum* with 24 in the pollen grain of 4–39–2–2.

A study was made of the chromosome behavior in the pollen mother cells of anthers imbedded in paraffin, sectioned 10 μ thick, and stained with crystal violet.

Meiosis in the hybrids was irregular. At diakinesis various chromosome groupings, including rings of four or more chromosomes, long chains of terminally united chromosomes, and what appeared to be tetravalent chromosomes were seen.

At metaphase I, 6 of the 61 cells examined in side view appeared to be normal, with all the chromosomes lined up on the metaphase plate. No univalents or trivalents could be detected. Fifty-five cells, or 90 percent, had one or more groups of nonoriented chromosomes, as shown in table 2. The greatest number of nonoriented chromosomes found in one cell was 6. These often showed signs of splitting, and they were frequently as large as the largest bivalents on the plate. Presumably, however, they were for the most part univalents rather than bivalents.

TABLE 2.—*Pollen mother cells in the F_1 hybrid showing nonoriented chromosomes at metaphase I and II, lagging chromosomes at anaphase I and II, and micronuclei at telophase II, with the number of chromosomes or chromosome groups involved in each cell*

Item	Cells showing described behavior in indicated number of chromosomes									Total cells
	Number chromosome involved									
	0	1	2	3	4	5	6	7	8	
	No.	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*
Metaphase I: Cells showing nonoriented chromosomes	6	22	20	9	1	2	1	----	----	61
Anaphase I: Cells showing lagging chromosomes	----	9	36	7	20	2	4	1	2	81
Metaphase II: Cells showing nonoriented chromosomes	21	23	19	11	4	5	4	1	----	88
Anaphase II: Cells showing lagging chromosomes	190	18	15	3	----	----	----	----	----	226
Telophase II: Cells showing micronuclei	76	27	15	3	1	----	----	----	1	123

Trivalent associations were extremely rare either on the metaphase plate or among the nonoriented chromosomes, though a few were seen, as well as one chain of four. Nonoriented groups of two or three chromosomes were occasionally seen.

At anaphase I, all of the 81 cells examined showed lagging chromosomes. In some cases the lagging chromosome was clearly a univalent,

and rarely a lagging trivalent was found, but most of the lagging chromosomes had the appearance of conjugated pairs. Presumably these last were univalents which were splitting. The number of lagging chromosomes or chromosome groups found in each of the 81 cells examined in side view is given in table 2.

At telophase I some of the lagging chromosomes had a faded appearance and seemed to be disintegrating in the cytoplasm. At interkinesis the chromosomes were well separated, with no suggestion of secondary association.

At metaphase II a somewhat smaller proportion of the cells showed nonoriented chromosomes than at metaphase I, as is shown in table 2. Some of the chromosomes which lay outside the spindle at the first division apparently either were recovered at interkinesis or had disintegrated before the second division.

Few lagging chromosomes were observed at anaphase II, but there were numerous nonoriented chromosomes scattered throughout the cells, often near the cell walls. Forty-six cells in a total of 226 observed showed nonoriented chromosomes. The number of lagging chromosomes found in these same 226 cells, or 452 division figures, is shown in table 2.

Micronuclei were counted at telophase II, and the number of micronuclei found in each of the 123 cells observed is shown in table 2. There were surprisingly few, considering the number of nonoriented and lagging chromosomes observed at earlier stages. Probably many of the chromosomes which were cast out during the first division or in early stages of the second division were recovered at anaphase of the second division, and it is possible that at least some of the nonoriented chromosomes were lost through degeneration. The latter possibility is supported by the fact that there were no micronuclei to be found in the quartets or young pollen grains. It is unlikely that micronuclei present at telophase II would become reincorporated into the pollen nuclei.

Pollen resulting from these irregular divisions varied considerably in size, the smallest grains making up 5 to 10 percent of the total. These smallest grains and some of the medium-sized grains were empty or contained partially disintegrated protoplasm. The largest grains, making up approximately one-third of the total, had the dense and evenly granulated protoplasm characteristic of good pollen. Stainability with acetocarmine of the pollen of some of the F_1 plants is shown in table 3. It was fairly high, considering the numerous irregularities found in meiosis, ranging from 14.8 to 30.8 percent in the field at University Farm, from 23.9 to 45.2 percent in the greenhouse, and above 50 percent in the field at Castle Danger on the north shore of Lake Superior, where environmental conditions usually favor flowering and fruit setting in the potato. In the greenhouse there was as much variation between different pots of an F_1 clone as between different clones.

The relationship of pollen stainability to germination on agar is shown in table 4. Flowers from each of three different F_1 plants and from *Solanum demissum* were used. Pollen of each flower was first shaken onto an agar gel containing 1 gram of agar and 20 grams of sugar to 100 cc of water. The remaining pollen was examined in acetocarmine. In the *S. demissum* flower 61.3 percent of the pollen was stainable and

26.8 percent germinated, indicating that nearly half of the stainable pollen was viable on agar. Pollen of the F_1 plants behaved very differently, though some pollen of each flower germinated. Stainability averaged 59.3 percent, and germination only 2.3 percent. Grains that germinated were all of the largest size.

TABLE 3.—*Stainable pollen in different F_1 plants of Solanum demissum × S. tuberosum selection 4–39–2–2*

Location, date, and plant breeding No.	Flowers	Mean percent of stainable pollen [1]	Location, date, and plant breeding No.	Flowers	Mean percent of stainable pollen [1]
University Farm, summer of 1933:	*Number*	*Percent*	Greenhouse, Mar. 5 to 17, 1934—Continued.	*Number*	*Percent*
1	3	20.0±6.1	7, pot b	10	31.1±2.0
4	5	30.8±2.8	7, pot c	13	32.1±1.5
8	3	29.0±8.7	7, pot d	11	34.5±2.7
9	5	14.8±2.1	10, pot a	7	28.7±4.6
10	2	29.5±7.5	10, pot b	7	37.0±3.5
11	2	26.5± .5	10, pot c	4	37.7±1.4
Greenhouse, Mar. 5 to 17, 1934:			10, pot d	6	45.2±1.4
3	15	23.9±2.4	Castle Danger, July 27, 1934:		
4	1	31.0±	12	5	56.0±5.0
5, pot a	11	28.5±3.0	13	5	55.0±4.4
5, pot b	7	33.4±3.8	14	5	50.4±3.7
7, pot a	9	24.0±2.2			

[1] 100 pollen grains of each flower were counted. Standard error is used rather than probable error.

TABLE 4.—*Relation between stainability of the pollen of Solanum demissum and its hybrids and its germination on agar*

Item	Stainable grains		Total	Germinated grains		Total
	Number	*Percent*	*Number*	*Number*	*Percent*	*Number*
S. demissum	676	61.3	1,102	327	26.8	1,219
F_1, flower 1	170	68.0	250	8	1.6	500
F_1, flower 2	158	63.2	250	8	1.6	500
F_1, flower 3	117	46.8	250	18	3.6	500
F_1 mean		59.3			2.3	

Most of the F_1 plants did not set selfed seed, although numerous hand pollinations were made both in the greenhouse and in the field at University Farm. The only plant that set selfed seed in the greenhouse was plant 15, which was also the only one that died without forming tubers. Plants 1 to 11 were grown at University Farm in 1934 and did not set selfed seed. Plants 12, 13, and 14 were moved from the greenhouse to the potato-breeding plots at Castle Danger, where they grew vigorously and blossomed freely, having a succession of flowers throughout the season. The pollen was over 50 percent stainable. Plant 12 was estimated to have produced seed from not more than 1 percent of its flowers, and plant 13 was even less fruitful. Plant 14, which bloomed most profusely, set almost no seed. The naturally set seed of plants 12 and 13 was used to grow the F_2 families 12 and 13.

THE F_2 FAMILIES

In 1934 F_2 families 12 and 15 were grown from seed of F_1 plants 12 and 15. The seed from which F_2 family 15 was grown was produced in the greenhouse by self-pollination. The 103 plants of F_2

family 15 were uniform and practically indistinguishable from the *Solanum demissum* parent. No tubers were obtained from this family, but 90 of the plants set seed. The 90 F_3 families grown in 1936 were also practically indistinguishable from *S. demissum*.

F_2 family 12 was grown from seed obtained from the naturally set fruit of F_1 plant 12. Chromosome counts were obtained from the root tips of 15 of the plants of this family. None of the plants had more than 58 chromosomes, 2 less than the number in the F_1. There was no apparent relationship between chromosome number and either tuber formation or seed production. The approximate chromosome counts of those that set seed as well as tubers were 49, 50, 51, and 56; of those forming only tubers but no seed 50, 53, 54, 56, and 58; and of those forming neither tubers nor seed 48, 52, 53, 54, 56, and 58.

In 1936 F_2 family 13 and a further lot of F_2 family 12 were grown from seed of naturally set fruit produced at Castle Danger by F_1 plants 12 and 13. These two families were similar to each other and resembled *S. tuberosum* in general appearance. Several morphological and physiological characters were measured. The results are shown in tables 5 to 20.

MORPHOLOGICAL CHARACTERS

The range in cotyledon width is shown in table 5. Cotyledons of the F_2 plants varied from narrower than those of *Solanum demissum* to as wide as the widest of *S. tuberosum*. The range in cotyledon length is shown in table 6. Cotyledons of the F_2 plants varied from shorter to longer than the cotyledons of the two parent families. The larger standard deviations show that this greater range was due to greater variability in cotyledon length among the F_2 plants and not to the fact that more F_2 than parent plants were measured. The range in shape indices, width divided by length, is shown in table 7. Indices of the F_2 plants ranged from as low as the lowest index of *S. demissum* plants to as high as the highest index of plants of *S. tuberosum*, with the mean indices half way between the means of the parent families. This distribution is to be expected if the character is determined by a number of factors.

TABLE 5.—*Distribution of individuals in the parent and F_2 families according to cotyledon width*

Family	Plants having cotyledons of a width—								Total plants	Mean width of cotyledons [1]
	1 mm.	2 mm.	3 mm.	4 mm.	5 mm.	6 mm.	7 mm.	8 mm.		
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Millimeters*
Solanum demissum		2	20	8	1				31	3.5±0.1
S. tuberosum 4-39-2-2		1	2	1	2	14	9	2	31	6.0± .3
F_2 12	2	2	7	19	14	10	1	1	56	4.4± .2
F_2 13	5	3	7	14	15	5	1		50	4.0± .2

[1] Standard error is used rather than probable error.

Terminal leaflets of both F_2 families were larger than those of either of the parents, as is shown in table 8, but smaller than those of F_1 plants. Shape indices of the terminal leaflets, shown in table 9,

ranged from the lowest of *Solanum demissum* to almost the highest of *S. tuberosum* with the means half way between the means of the parents, as was true of the cotyledon shape indices.

TABLE 6.—*Distribution of individuals in the parent and F_2 families according to cotyledon length*

Family	Plants having cotelydons of a length of—											Total plants	Mean length of cotyledons [1]	Standard deviation
	2 mm.	3 mm.	4 mm.	5 mm.	6 mm.	7 mm.	8 mm.	9 mm.	10 mm.	11 mm.	12 mm.			
	No.	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*Number*	*Millimeters*	
Solanum demissum	----	----	----	2	6	3	7	6	4	3	----	31	8.1±0.3	1.8
S. tuberosum 4-39-2-2	----	----	2	2	3	4	8	7	2	3	----	31	7.9± .3	1.8
F_2 12	2	1	1	4	8	11	11	6	6	4	2	56	7.5± .3	2.3
F_2 13	3	3	3	7	8	3	8	2	7	2	4	50	7.1± .4	2.7

[1] Standard error is used rather than probable error.

TABLE 7.—*Distribution of individuals in the parent and F_2 families according to cotyledon shape*

Family	Plants having indicated shape index (cotyledon width divided by length)													Total plants	Mean shape index of cotyledons [1]
	0.40	0.45	0.50	0.55	0.60	0.65	0.70	0.75	0.80	0.85	0.90	0.95	1.00		
	No.	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*Number*	
Solanum demissum	9	13	7	----	1	----	----	----	----	----	----	----	----	30	0.44±0.01
S. tuberosum 4-39-2-2	----	----	1	----	1	1	4	10	3	1	5	----	3	29	.76± .03
F_2 12	1	3	12	4	14	6	7	3	1	2	2	----	----	55	.60± .02
F_2 13	3	1	15	1	9	5	5	3	3	2	1	----	2	50	.61± .02

[1] Standard error is used rather than probable error.

TABLE 8.—*Terminal leaflet length and width of the parent families, a group of F_1 plants, and the F_2 families*

Family	Leaflets measured	Mean width [1]	Mean length [1]	Family	Leaflets measured	Mean width [1]	Mean length [1]
	Number	*Millimeters*	*Millimeters*		*Number*	*Millimeters*	*Millimeters*
Solanum demissum	27	11±1	18± 1	F_2 12	50	32±1	45±2
S. tuberosum 4-39-2-2	20	25±2	33± 2	F_2 13	42	28±2	40±2
F_1	15	35±7	53±10				

[1] Standard error is used rather than probable error.

TABLE 9.—*Distribution of individuals of the parent and F_2 families and a group of F_1 plants according to terminal-leaflet shape*

Family	Plants having indicated shape index (Terminal leaflet width over length)										Total plants	Mean shape index of terminal leaflets [1]
	0.50	0.55	0.60	0.65	0.70	0.75	0.80	0.85	0.90	1.00		
	No.	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	
Solanum demissum	1	2	8	8	4	3	1	----	----	----	27	0.63±0.01
S. tuberosum 4-39-2-2	----	----	1	1	2	3	5	5	2	1	20	.78± .02
F_1	----	----	3	6	4	2	----	----	----	----	15	.65± .01
F_2 12	1	----	6	6	9	15	9	2	1	1	50	.70± .01
F_2 13	2	2	6	5	6	13	3	4	1	----	42	.69± .01

[1] Standard error is used rather than probable error.

Areas of mature compound leaves were measured in square centimeters. Leaves of both F_2 families were larger on an average than those of the *Solanum tuberosum* parent, though considerably smaller than those of the F_1 plants, and the range was from the smallest leaves of *S. demissum* to almost twice the size of the largest leaves of the *S. tuberosum* parent, as shown in table 10. Leaves of *S. tuberosum* family 4–39–2–2 were of medium size for the species.

TABLE 10.—*Distribution of individuals of the parent and F_2 families and a group of F_1 plants according to leaf size*

Family	Plants having leaves with an area of—												Total plants	Mean area of leaves [1]
	10 cm.²	20 cm.²	30 cm.²	40 cm.²	50 cm.²	60 cm.²	70 cm.²	80 cm.²	10 cm.²	100 cm.²	110 cm.²	120 cm.²		
	No.	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*Cm.²*
Solanum demissum	26												26	6.4±0.5
S. tuberosum 4–39–2–2		3	9	7	1								20	28.5±4.0
F_1							2	2	3	2	2	2	13	45.1±2.0
F_2 12	5	8	11	8	7	5	2	1	1				48	37.7±2.9
F_2 13	10	8	5	4	9	2	1	1					40	32.2±3.1

[1] Standard error is used rather than probable error.

Several stem characters were measured. The parents differed markedly in height, and, while each parent family varied considerably in stem length, there was no overlapping on a frequency distribution. The F_2 families showed a range in height from next to the shortest plants of *Solanum demissum* to approximately the mean height of the *S. tuberosum* parent, as shown in table 11. In this character, and also in internode length, shown in table 12, the F_2 families tended to be intermediate between the two parents, and were slightly shorter than the F_1 plants. In stem diameter at ground level, shown in table 13, the means of the F_2 families were intermediate between those of the parents and less than the mean of the F_1. More than half of the F_2 plants resembled the *S. tuberosum* parent in having short stolons, but there were some plants in each family that resembled *S. demissum* in having long stolons, as is shown in table 14.

TABLE 11.—*Distribution of individuals of the parent and F_2 families and a group of F_1 plants according to stem height*

Family	Plants having a stem height of—												Total plants	Mean stem height [1]
	2 inches	4 inches	6 inches	8 inches	10 inches	12 inches	14 inches	16 inches	18 inches	20 inches	22 inches	24 inches	26 inches	
	No.	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*In.*
Solanum demissum	1	2	11	10	3									27	6.5±0.4
S. tuberosum 4–39–2–2						2	2	1	5	3	3	2	3	21	18.7±1.0
F_1			1	3	6	4	1							15	9.5± .5
F_2 12		2	8	11	13	8	2	3	1	1				49	9.2± .5
F_2 13		5	10	13	4	4	3	1	1					41	8.1± .5

[1] Standard error is used rather than probable error.

TABLE 12.—*Internode length of the F_2 families compared with internode length of the parent families and a group of F_1 plants*

Family	Plants measured	Mean internode length[1]	Family	Plants measured	Mean internode length[1]
	Number	*Mm.*		*Number*	*Mm.*
Solanum demissum	26	6.3±0.4	F_2 12	49	14.3± .9
S. tuberosum 4-39-2-2	21	28.1±1.2	F_2 13	41	12.4±1.0
F_1	15	19.1±1.2			

[1] Standard error is used rather than probable error.

TABLE 13.—*Distribution of individuals of the parent and F_2 families and a group of F_1 plants according to stem diameter*

Family	Plants having stems with a diameter of—																Total plants	Mean stem diameter[1]
	2.5 mm.	3.0 mm.	3.5 mm.	4.0 mm.	4.5 mm.	5.0 mm.	5.5 mm.	6.0 mm.	6.5 mm.	7.0 mm.	7.5 mm.	8.0 mm.	8.5 mm.	9.0 mm.	9.5 mm.	10.0 mm.	10.5 mm.	
	No.	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*Mm.*
Solanum demissum	1	3	1	10	5	6												26	3.9±0.1
S. tuberosum 4-39-2-2					1	1	2		1	1		5	4	2	3	1		21	7.5± .3
F_1								2	2	2	1	5		3				15	7.4± .3
F_2 12	1		3	2	2	4	3	3	6	6	3	7	7	1		1	1	50	6.5± .3
F_2 13	4			5	4	7	6	4	1	2	1	5	2	3				44	5.4± .3

[1] Standard error is used rather than probable error.

TABLE 14.—*Distribution of individuals of the parent and F_2 families and a group of F_1 plants according to stolon length*

Family	Plants having stolon length of—											Total plants	Mean stolon length[1]
	2 cm.	4 cm.	6 cm.	8 cm.	10 cm.	12 cm.	14 cm.	16 cm.	18 cm.	20 cm.	22 cm.		
	No.	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*Centimeters*
Solanum demissum												18	30.0
S. tuberosum 4-39-2-2	13	3	4									20	2.0±0.4
F_1												15	25.0
F_2 12	10	10	4	3	2	3	2	3		2	2	41	7.4±1.1
F_2 13	14	10	5	2	3	1	1		1	1	1	39	5.9±1.1

[1] Standard error is used rather than probable error.

The F_2 families set fewer tubers than their cultivated parent and far fewer than the F_1 plants, as shown in table 15. They also showed less variation in number of tubers set than did the cultivated parent. Both F_2 families and the cultivated parent showed great variation in yield. The total weight of all tubers of plants which set a crop ranged from 1 to 420 grams per plant, as shown in table 16, with the mean yields of the F_2 families nearly as great as the mean yield of the cultivated parent. Segregation for size of tuber was quite different from segregation for total yield, as may be seen by comparing table 17 with table 16. While none of the tubers of *Solanum demissum* weighed over 2 grams, and only 1 plant of the *S. tuberosum* parent produced a

tuber weighing more than 20 grams, 8 of the 14 F_1 plants, and 22 of the 78 F_2 plants produced tubers weighing more than 20 grams.

TABLE 15.—*Distribution of individuals of the parent and F_2 families and a group of F_1 plants according to the number of tubers set*

Family	Plants having indicated number of tubers											Total plants	Mean number of tubers [1]	Standard Deviation
	3	8	13	18	23	28	33	38	43	48	Over 50			
	No.	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	
Solanum demissum	11	5	----	1	----	1	----	----	----	----	----	18	7±3	8
S. tuberosum 4-39-2-2	7	1	1	3	1	1	4	1	1	2	2	24	24±5	20
F_1	----	----	----	----	4	1	4	2	1	----	3	15	40±4	----
F_2 12	13	7	6	6	5	2	2	2	----	----	----	43	14±2	10
F_2 13	8	12	4	4	2	4	1	2	----	2	----	39	16±2	13

[1] Standard error is used rather than probable error.

TABLE 16.—*Distribution of individuals of the parent and F_2 families and a group of F_1 plants according to yield*

Family	Plants producing a crop that weighed—												Total plants	Mean crop weight [1]
	20 gm.	50 gm.	80 gm.	110 gm.	140 gm.	170 gm.	200 gm.	230 gm.	260 gm.	290 gm.	320 gm.	350 gm.	Over 365 gm.	
Solanum tuberosum 4-39-2-2	*No.* 11	*No.* 4	*No.* 1	*No.* 3	*No.* 1	*No.* 1	*No.* 1	*No.* 1	*No.* ----	*No.* ----	*No.* ----	*No.* 1	*No.* ------	*No.* 24	*Grams* 86±22
F_1	----	----	----	----	1	2	3	2	2	2	1	----	1	14	238±16
F_2 12	14	9	6	5	----	1	1	1	----	2	1	----	2	42	85±16
F_2 13	12	9	6	3	1	1	1	----	----	----	1	----	------	34	58±11

[1] Standard error is used rather than probable error.

TABLE 17.—*Distribution of individuals of the parent and F_2 families and a group of F_1 plants according to weight of largest tuber*

Family	Plants the largest tuber of which weighed within the indicated class limits											Total plants	Mean tuber weight [1]
	3 gm.	8 gm.	13 gm.	18 gm.	23 gm.	28 gm.	33 gm.	38 gm.	43 gm.	48 gm.	53 gm.	Over 55 gm.		
	No.	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*Grams*
Solanum demissum	26	----	----	----	----	----	----	----	----	----	----	----	26	1
S. tuberosum 4-39-2-2	10	6	1	6	----	----	----	----	----	----	----	1	24	11±3
F_1	----	----	1	5	4	4	----	----	----	----	----	----	14	21±1
F_2 12	14	4	3	8	4	3	3	----	----	1	2	1	43	18±2
F_2 13	11	9	4	3	2	2	----	3	----	----	1	----	35	13±3

[1] Standard error is used rather than probable error.

PHYSIOLOGICAL CHARACTERS

Such physiological characters as heat injury, frost injury, and maturity are of great importance to the potato grower. Because direct measurements of these characters are difficult, arbitrary scales were used.

The season of 1936 was extremely hot and dry, affording an excellent opportunity to study heat and drought injury. Among the F_2 plants it ranged from none to lethal injury, as shown in table 18. The

means were closer to the mean of the tuberosum than the demissum parent. The later renewal of growth by means of underground stolons, characteristic of *Solanum demissum*, was not found among the F_2 plants. Immaturity was recorded on September 27 on a scale of 0 to 5. At this time all plants of *S. demissum* and all the F_1 plants were still green, while all but two of the plants of the *S. tuberosum* parent were mature. The F_2 families showed a complete range from mature to green, as shown in table 19, the means falling halfway between the parent means. Frost-injury notes were taken on October 1 only on plants that were quite immature. Plants of *S. demissum* showed no injury from the light frost, while the two plants of the *S. tuberosum* parent that were still green were killed. Plants of the F_2 families showed a complete range from no injury to lethal injury, as shown in table 20, with 5 of the 54 plants on which frost notes were taken showing no injury. These five plants were too late in maturing to be of much use in northern latitudes, but it is probable that some of the F_2 plants which had matured before the frost struck possessed as much frost hardiness as these five plants.

TABLE 18.—*Distribution of individuals of the parent and F_2 families and a group of F_2 plants according to relative heat and drought injury*

Family	Plants showing indicated amount of injury				Total plants	Mean [1] injury
	None, 0	Medium, 1	Severe, 2	Dead, 3		
	Number	*Number*	*Number*	*Number*	*Number*	
Solanum demissum		9	17	5	31	1.87±0.12
S. tuberosum 4-39-2-2	12	9	1	6	28	1.04± .22
F_1					15	.00± .00
F_2 12	9	31	10	6	56	1.23± .11
F_2 13	17	17	10	9	53	1.21± .15

[1] Arbitrary scale. Standard error is used rather than probable error.

TABLE 19.—*Distribution of individuals of the parent and F_2 families and a group F_1 plants according to immaturity on September 27*

Family	Plants showing immaturity [1]						Total plants	Mean [2] immaturity
	0	1	2	3	4	5		
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	
Solanum demissum						26	26	5.0±0.0
S. tuberosum 4-39-2-2	20			2			22	.3± .2
F_1						15	15	5.0± .0
F_2 12	12	9	5	5	7	11	49	2.4± .3
F_2 13	12	7	6	4	9	7	45	2.3± .3

[1] On a scale of 0 for dead to 5 for all green.
[2] Arbitrary scale. Standard error is used rather than probable error.

Leaflets of the F_2 plants were shaped more like those of *Solanun tuberosum* than were the leaflets of the F_1 plants. Because of the greater vigor of the F_1 plants, their leaflets were longer and wider, and the mature leaf area was larger than in the F_2 plants. In stem height and diameter the F_2 plants were slightly smaller and more like *S. demissum* than the F_1 plants, and in internode length the F_2 were

definitely shorter and more like *S. demissum* than the F_1 plants. Stolon length was shorter and more like *S. tuberosum* in the F_2 than in the F_1 plants. Tubers of the F_2 were smaller and far fewer in number and the yield was far less than in the F_1 plants. In physiological characters the F_2 plants showed some heat injury whereas the F_1 did not, the F_2 were much earlier in maturing, and the F_2 plants were not quite as frost-hardy as the F_1.

TABLE 20.—*Distribution of individuals of the parent and F_2 families and a group of F_1 plants according to frost injury*

Family	Plants showing injury rated as [1]—						Total plants	Mean injury [2]
	0	1	2	3	4	5		
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	
Solanum demissum	26						26	0 ±0.0
S. tuberosum 4-39-2-2						2	2	5.0± .0
F_1	3	4	3	3	1	1	15	1.9± .4
F_1 12	3	3	6	4	7	5	28	2.9± .3
F_2 13	2	3	1	6	3	11	26	3.5± .3

[1] On a scale of 0 to 5. (5= lethal injury).
[2] Arbitrary scale. Standard error is used rather than probable error.

BACKCROSS FAMILIES

Four of the F_1 plants were backcrossed to *Solanum tuberosum*, with plants 3, 5, 7, and 10 as the pistillate parents, and Minnesota selection 40–4–2–6, a fourth-generation inbred selection of the U. S. D. A. 38946, as the pollen parent. The F_2 families and the backcross families are compared in table 21. In leaflet shape, stem diameter, stolon length, number of tubers, immaturity, and frost injury, means of both F_2 families and of all four backcross families were intermediate between the means of the parents. In length, width, and shape of the largest simple leaf, means of the F_2 and backcross families were approximately equal. In cotyledon length, in which the parents were very nearly equal, means of the F_2 families and three of the four backcross families were smaller than the means of the parents. In size of largest tuber, the means of the F_2 families and the backcross families were approximately equal, surpassing both parents. The yield of the backcrosses were slightly greater than the F_2 families. In leaflet length and width and leaf area, the means of the F_2 families were larger than the means of the backcross families and larger than those of either original parent. The F_2 families were less injured by heat than the backcross families and were more similar to *S. tuberosum* families in this respect. They were more like *S. demissum* than the backcross families in cotyledon width and shape index, stem height, and internode length.

DISCUSSION AND CONCLUSIONS

Of the 15 F_1 plants grown from the cross between *Solanum demissum* and *S. tuberosum*, 1 set fruit with viable seed in the greenhouse but produced no tubers. The breeding behavior of this plant in the F_2 and F_3 generations suggests that it was not a hybrid but a specimen of *S. demissum*.

TABLE 21.—*Characteristics of the four backcross families as compared with those of F_2 families 12 and 13* [1]

Families	Plants	Morphological characters										
		Cotyledon			Simple leaf			Leaflet			Mean leaf area
		Mean width	Mean length	Mean shape index	Mean width	Mean length	Mean shape index	Mean width	Mean length	Mean shape index	
	Number	*Millimeters*	*Millimeters*		*Millimeters*	*Millimeters*		*Millimeters*	*Millimeters*		*Square centimeters*
F_2 12	56	4.4±0.2	7.5±0.3	0.60±0.02	23.1±0.6	30.8±0.7	0.75±0.01	32±1	45±2	0.70±0.01	38±3
F_2 13	50	4.0± .2	7.1± .4	.61± .02	19.1± .7	26.7± .8	.72± .01	28±2	40±2	.69± .01	32±3
Backcross:											
40	40	5.0± .2	8.3± .3	.61± .02	19.1±1.0	25.2±1.3	.76± .01	28±2	38±2	.71± .02	32±2
41	15	4.4± .3	6.7± .5	.70± .03	19.3±1.5	25.7±2.0	.76± .02	19±1	31±2	.61± .03	29±3
42	70	4.8± .1	6.9± .2	.70± .02	20.4± .5	26.9± .7	.77± .01	22±1	33±1	.68± .02	32±2
43	50	4.5± .2	6.4± .2	.73± .02	19.4± .8	26.5±1.1	.73± .01	20±2	31±2	.63± .02	25±2

Families	Plants	Morphological characters							Physiological characters		
		Stem		Mean internode length	Mean stolon length	Mean number of tubers	Mean weight of largest tuber	Mean yield	Mean heat injury [1]	Mean immaturity [2]	Mean frost injury [3]
		Mean height	Mean diameter								
	Number	*Inches*	*Millimeters*	*Millimeters*	*Centimeters*	*Number*	*Grams*	*Grams*			
F_2 12	56	9.2±0.5	6.5±0.3	14±1	7.4±1.1	14±2	18±2	85±16	1.2±0.1	2.4±0.3	2.9±0.3
F_2 13	50	8.1± .5	5.4± .3	12±1	5.9±1.1	16±2	13±3	58±11	1.2± .1	2.3± .3	3.5± .3
Back cross:											
40	40	12.1± .6	5.6± .3	18±1	9.3±1.4	14±2	19±4	82±18	1.4± .1	2.3± .3	4.4± .2
41	15	13.5±1.3	5.2± .4	21±2	4.6±1.4	14±4	13±3	60±21	1.6± .2	2.3± .5	4.6± .2
42	70	11.7± .6	4.9± .2	18±1	9.0±1.0	21±3	19±2	123±18	1.5± .1	3.1± .2	3.6± .2
43	50	11.6± .7	4.9± .2	19±1	7.4± .9	14±2	21±6	85±16	1.4± .1	3.5± .3	3.3± .2

[1] Standard error is used rather than probable error.
[2] Arbitrary scale from 0 to 3.
[3] Arbitrary scale from 0 to 5.

The remaining 14 plants appeared to be true hybrids, and chromosome counts in some of them showed the presence of the expected 60 chromosomes. In the greenhouse they flowered but did not set selfed seed, though they set moderately well when backcrossed to *Solanum tuberosum*. The presence of a sufficient supply of viable pollen for self fertilization was indicated by pollen stainability of 24 to 45 percent in the greenhouse. and over 50 percent at Castle Danger. Since the work of Krantz et al. (*9a*) has shown that pollen that is 20 to 50 percent stainable cause sseed production in 76 to 94 percent of the plants of *S. tuberosum*, it was assumed that because of the proportion of stainable pollen found in the F_1 plants, some of them would produce seed. Despite this moderate amount of stainable pollen, viability on the agar medium used was only 2 percent, but even this low proportion of functional pollen among the many thousands of grains in each flower would insure a sufficient supply for seed production.

Artificial self-pollination in the greenhouse and in the field at University Farm failed to produce fruit setting. Three of the F_1 plants were moved to an environment more favorable to seed production, where they were highly vegetative and bloomed profusely throughout the summer. Of the three, plant 12 set a moderate amount of fruit and had the most seeds in each fruit. Plant 13 had fewer fruits, less seed in each fruit, and a lower rate of germination of seed. Plant 14, which bloomed the most profusely, set very few fruits, and the few seeds obtained failed to germinate.

Chromosome counts in 15 plants of F_2 family 12 were lower than would be expected if 48 of the 60 chromosomes in the F_1 plants paired into bivalents and the 12 chromosomes of the fifth genom were distributed at random. Ovules and pollen grains would then contain between 24 and 36 chromosomes each, and if all gametes were equally viable, the counts in the F_2 would range from 48 to 72, with a mean of 60. Irregularities seen in pollen formation indicate that some of the chromosomes were lost in meiosis, and the low chromosome counts in the F_2 plants could be explained wholly on this basis. A more probable explanation is the fertilization of ovules containing 24 to 36 chromosomes with pollen containing 24 chromosomes. Since in many genera pollen with extra chromosomes is not functional, it is possible that only the 24-chromosome pollen of the F_1 plants was functional. Chromosome counts of 48 to 60 could thus be obtained by self pollination of the F_1 plants, as well as by backcrossing the F_1 plants with the 24-chromosome pollen of *Solanum tuberosum*.

Segregation in the progenies of plants 12 and 13 showed a range from the extremes of *Solanum demissum* to the extremes of *S. tuberosum* selection 4–39–2–2, though most of the F_2 plants were more like *tuberosum* than were the F_1 plants. Both F_2 families 12 and 13 had larger leaves, larger terminal leaflets, and larger tubers than those of either parent.

The data show that ovules of the F_1 plants are moderately fertile but that the pollen is sparingly viable, and self-fertilization of the F_1 is either difficult or impossible. The F_2 families obtained do not differ markedly from the backcross families, and it appears that the naturally set seed of F_1 plants 12 and 13 may have been the result of accidental backcrossing with *Solanum tuberosum*.

SUMMARY

Inheritance of some characters in the interspecific cross *Solanum demissum* Lindl. × *S. tuberosum* L., was studied.

Solanum demissum is a small, prostrate, late-maturing species with narrow cotyledons, small leaves, long stolons, and many small tubers. It has been reported to have 72 chromosomes. *S. tuberosum* selection 4–39–2–2, the pollen parent is a large, erect, early-maturing, frost-tender selection with wide cotyledons, medium-sized leaves, short stolons, and many medium-sized tubers. *S. tuberosum* has been reported to have 48 chromosomes.

The 60-chromosome hybrids between those species showed considerable irregularity in meiosis, nonoriented and lagging chromosomes being usual. Fifteen plants were raised, of which three were fertile enough to produce F_2 families.

One F_2 family was practically identical with *Solanum demissum* in morphological characters and was uniform.

The other two F_2 families approached *Solanum tuberosum* in morphological characters and were variable. Chromosome counts in 15 of the plants of one of these families ranged from 48, the number in *S. tuberosum*, to 58, or 2 less than the number in the F_1 plants.

In cotyledon measurements, stem diameter, heat injury, maturity, frost injury, stolon length, number of tubers, and weight of the largest tuber, these F_2 families showed a range from the extreme of one original parent to the extreme of the other, with the means falling halfway between the parent means. Height of mature plants and internode length of the F_2 families resembled *Solanum demissum* more than 4–39–2–2, while the weight of the crop was similar to that of the cultivated parent. Terminal leaflets and mature leaves were larger than those of either parent.

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EFFECT OF TEMPERATURE ON THE RATE OF DETERIORATION OF FRESH VEGETABLES[1]

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INTRODUCTION

In recent years many improvements have been made in methods of handling fresh vegetables. Cold-storage rooms are being used extensively for long- and short-time storage, and shipments to distant markets are made in refrigerated cars or refrigerated trucks, the load frequently being precooled before it leaves the shipping point. All these practices are based on the well-recognized fact that the biological processes responsible for the break-down of the produce are greatly retarded by a lowering of the storage temperature. This fact is emphasized by the results of storage experiments carried out with many different vegetables. Generally, a lowering of the storage temperature has resulted in a considerable lengthening of the storage life of a particular vegetable, provided the temperature was not so low as to cause injury from freezing or chilling.

While nearly all of these experiments indicate a close relationship between temperature and the rate of deterioration, few data are available which show in numerical terms exactly what this relationship is. In some cases only one or two different storage temperatures were used, and usually only the temperature of the room, instead of that of the vegetable, was recorded; no account being taken of the fact that many hours, sometimes days, elapse before the temperature of vegetables in a commercial package reaches equilibrium with the temperature of the surrounding atmosphere. While such experiments may solve specific, practical problems, they are of little value in establishing broad, general principles governing the relationship between temperature and storage life of different vegetables. If it were possible to deduce a general rule which would express this relationship with a reasonable degree of accuracy, such information should furnish a valuable basis for solving many practical problems involved in the storage and transportation of fresh vegetables.

Several difficulties present themselves in determining the relationship between temperature and rate of deterioration. Different factors, such as respiration, transpiration, translocation, and metabolic activity, may contribute to the break-down of vegetables in storage. In some vegetables, such as sweet corn and peas, a pronounced lowering of palatability results from the loss of sugar. Usually this process occurs at a rate which is much more rapid than wilting or other changes in the external appearance would indicate. Furthermore, the rate of sugar loss in vegetables is by no means uniform. Appleman and Arthur (*1*),[2] working with sweet corn, and Bisson, Jones, and Robbins

[1] Received for publication December 27, 1938. Paper No. 181, Department of Vegetable Crops, Cornell University. This study was financed in part by a grant from the Empire State Gas & Electric Association.

[2] Italic numbers in parentheses refer to Literature Cited, p. 58.

(*4*), in experiments with asparagus, showed that these vegetables, when held at a high temperature, lose sugar very rapidly at first. Later, after most of the sugar has been depleted the rate of loss is greatly reduced; in fact, the percentage of sugar may actually increase toward the end of the storage period as a result of hydrolysis of other carbohydrates.

In many leafy vegetables, the first symptom of deterioration is an excessive wilting of the tender tissues. This may or may not be associated with observed changes in chemical composition. In storage experiments with snap beans, Parker and Stuart (*8*) showed that the percentage composition changed only very little, even though some lots were held for several days at room temperature. Ultimate deterioration of snap beans is usually brought about by shriveling and by decay through the spread of disease organisms.

From these considerations, it becomes evident that the results of any study of the rate of deterioration will depend largely on the criterion employed in measuring the rate of break-down. Nevertheless, when different lots of vegetables have reached a point where they become entirely unsalable, their appearance is very nearly alike regardless of the temperature at which they were kept. Chlorophyll-bearing parts of the plant tissue begin to turn yellow, many of the plant cells have died, or collapsed, or reached the stage of permanent wilting; secondary infection with bacteria and fungi begins to spread rapidly. On approaching this condition a salable product may change into a decaying mass with surpising speed, sometimes within a few hours.

In spite of the difficulties outlined, it was felt worth while to carry out a study to determine the relation between temperature and rate of deterioration provided proper precautions were taken to obtain comparable data and provided the limitations of such experiments were fully recognized.

EXPERIMENTAL METHODS

All vegetables used in these experiments were either grown on nearby truck farms or in the gardens of the Department of Vegetable Crops at Cornell University, Ithaca, N. Y. In every case the experiments were started within 4 hours after the vegetables had been harvested. Only produce of good quality, free from disease injury, was used in these studies.

The storage experiments were carried out in the experimental cold-storage rooms of the Department of Vegetable Crops. Four rooms were used in which the temperature was held at 35°, 50°, 65°, and 80° F., respectively. The corresponding relative humidity in these rooms averaged 94, 82, 74, and 68 percent. Temperature fluctuations were less than 3° and the relative humidity varied not more than 5 percent. No attempt was made to maintain the same relative humidity in all the rooms since this would have presented considerable technical difficulty, and also because under conditions of commercial practice, the prevailing relative humidity is nearly always higher at the lower storage temperature, and vice versa.

The vegetables used in the experiments were peas (*Pisum sativum* L.), spinach (*Spinacia oleracea* L.). radish (*Raphanus sativus* L.), lettuce (*Lactuca sativa* L.), celery (*Apium graveolens* L.), brussels sprouts (*Brassica oleracea* var. *gemmifera* DC.), asparagus (*Asparagus officinalis* L.), and sweet corn (*Zea mays* var. *rugosa* Bonafous).

The vegetables were stored in commercial containers, that is, peas and spinach in 1-bushel baskets, radishes in one-half-bushel baskets, lettuce in crates containing 2 dozen heads, celery in two-thirds celery crates, sweet corn in bags containing 5 dozen ears, brussels sprouts in 1-quart baskets, and asparagus in 2-pound bunches. Duplicate lots were used for all vetetables.

Accurate temperature records of each lot were kept by placing two or three thermometers near the center of each container. Depending on the rate of temperature change within the containers, readings were taken every hour, every 2 or 3 hours, or twice daily. From these records, curves were drawn showing accurately the temperature changes in each lot.

At frequent intervals each lot was inspected for condition and careful notes were taken on the progress of deterioration. A special effort was made to determine as accurately as possible the time at which each lot became unsalable. With some practice it became possible to estimate the time required to reach this stage of break-down with sufficient accuracy so that two workers making observations independently would obtain results varying less than 7 percent of the total storage time.

For most vegetables the relative rate of break-down of different lots was determined by comparing the time required to render these lots totally unsalable. As was pointed out before, the palatability of some vegetables, particularly those with a high sugar content, is subject to a more rapid deterioration than their appearance would indicate. For that reason the rate of deterioration of sweet corn, peas, and asparagus was also determined by taking samples for chemical analysis at certain intervals. Analyses for sugars were made by standard methods, as described in methods of the Association of Official Agricultural Chemists (*3*).

METHOD OF CALCULATING RESULTS

The relative rate of break-down in vegetables at different temperatures may be expressed in two ways. Calculations may be based either on the temperature of the storage room or on the temperature of the vegetables themselves. As illustrated in figure 1, the temperature of a vegetable when stored in commercial containers may differ widely from that of the surrounding atmosphere. A lot of spinach, for instance, which had an initial temperature of 90° F. and was held at 35° did not reach temperature equilibrium until the end of 60 hours. Obviously the rate of deterioration was much more rapid during the first 60 hours than later. Correspondingly, the average temperature of another lot held at 80° was about 14° higher than that of the surrounding air as a result of heat evolved during rapid respiration. Consequently, entirely different results may be obtained depending on whether the temperature of the storage room or that of the vegetable itself is used as a basis for calculating the temperature coefficient.

Considering first the temperature of the storage room only, the experimental results were expressed in a series of curves (fig. 2) which indicate for each vegetable how long they can be held at the different storage temperatures before complete break-down occurs. Although these curves do not express the true relationship between temperature and rate of deterioration, they may become valuable in solving certain practical storage problems.

In order to establish the true relationship between temperature and the rate of break-down the temperature of the vegetable itself, rather than that of the room, had to be considered. First, it was necessary

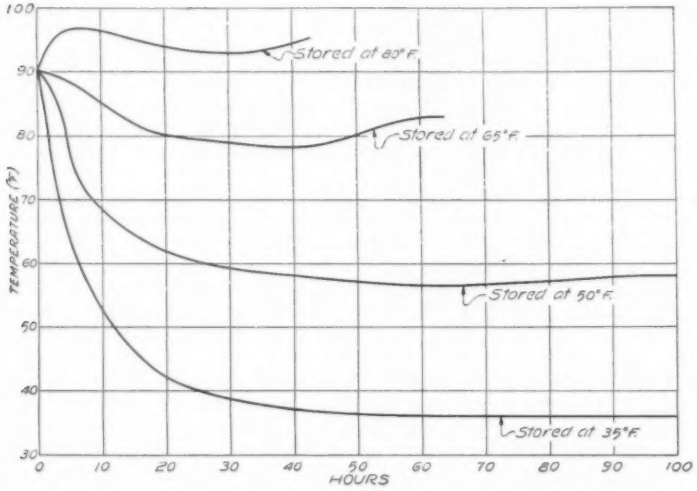

FIGURE 1.—Temperature inside the containers of four lots of spinach after transfer from 90° F. to rooms held at a constant temperature of 35°, 50°, 65°, and 80° respectively.

to calculate for each experimental lot a temperature coefficient which would express the rate of break-down at that particular temperature. The coefficient corresponding to a temperature of 32° F. was arbi-

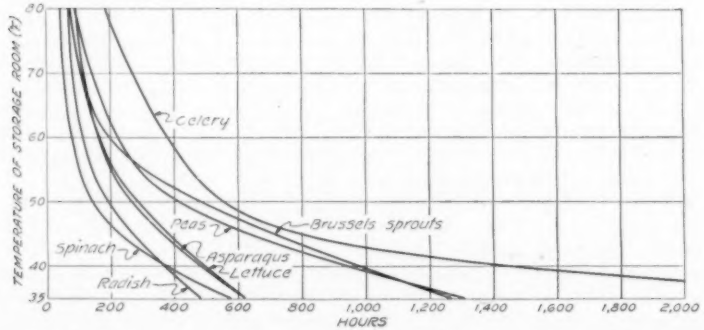

FIGURE 2.—Time-temperature curves based on the temperature of the storage rooms, indicating how long different vegetables can be held at a definite room temperature before complete deterioration occurs.

trarily set at 1. The coefficient for any other temperature then is a rate factor which indicates how much more rapidly deterioration occurs at that temperature than it does at 32°. Provided the temperature of each experimental lot is known and remains constant

throughout the storage period, the coefficient for each lot can easily be calculated by dividing the total storage time of a vegetable held at 32°, usually expressed in hours, by the total storage time of the particular lot in question. Total storage time in this case is the period from harvest until the vegetables have reached a stage at which they become unsalable. Assuming for instance that the storage life of a vegetable is 300 hours at an average temperature of 32° and 100 hours at 50°, the temperature coefficient corresponding to 50° would be 3. It also follows that the total storage time multiplied by the corresponding temperature coefficient must give a product which is a constant for any one vegetable. This may be expressed in the form of an equation $C_t \times h = K$, where C is the temperature coefficient corresponding to the temperature t, h the total storage time at that temperature, and K a constant. Since it was postulated that at a temperature of 32° the value for C_t is equal to 1, the value for K must be numerically equal to the storage time h at that temperature.

Because the average temperature of the experimental lots was never as low as 32° F., the total storage time for that temperature and the value for K had to be found by extrapolation. The calculation of the coefficients was further complicated by the fact that the temperature of each lot fluctuated over a fairly wide range, even though the room temperature remained constant. In addition, an inspection of the data indicated that the rate of deterioration increases geometrically with a rise in temperature. Obviously, a serious error would be introduced if one were to use the arithmetic mean of each temperature curve in these calculations, since too little weight would be given to the higher temperature values of the curves.

No simple method was available by which the temperature coefficient could be calculated directly from the available data because of the complications mentioned. It was possible, however, to arrive at fairly accurate values by a method which in principle consists of making a series of approximations to find a curve which gives the correct coefficients for each vegetable at different temperatures. All calculations were based on the supposition made earlier that for every lot of vegetables the product $C_t \times h$ must be the same and equal a constant K. The correctness of each curve could then be tested by inserting values for time and temperature as determined experimentally. Theoretically the curve would be perfect if the product $C_t \times h$ would be exactly the same for each one of the four lots of the same vegetable. How these calculations were carried out in detail can best be explained by using an example.

Figure 1 shows the temperature curves for four lots of spinach held at 35°, 50°, 65°, and 80° F., respectively. The total storage time required to render each lot unsalable is given in table 1.

TABLE 1.—*Rate of deterioration in spinach at four different storage temperatures*

Item	Data for room temperature of—			
	35° F.	50° F.	65° F.	80° F.
Total storage life, hours	576	141	64	43
Rate ratio	1	4.09	9.00	13.40
Values for K as calculated from curve b [1]	748.8	536.8	643.0	741.1
Values for K as calculated from curve c [1]	845.9	842.7	840.4	849.1

[1] Of figure 3.

In order to establish a preliminary rate curve which could later be corrected by the method of approximations it was necessary to disregard temporarily the error which results from basing calculations on the arithmetic mean of the temperature curves. Thus the arithmetic means of the temperature curves for the 35° and 80° F. lots were determined to be about 38° and 94.5° while the corresponding storage periods for these two lots were 43 and 576 hours, respectively. In other words, the rate of break-down of the first lot was $\frac{576}{43}$ or 13.4 times more rapid than that of the second one. Assuming that the rate increment between these two temperatures is a simple logarithmic function, approximate values for the coefficients in the intermediate temperature range could be found by the graphic method. The temperature was plotted on the abscissa on an algebraic scale and the

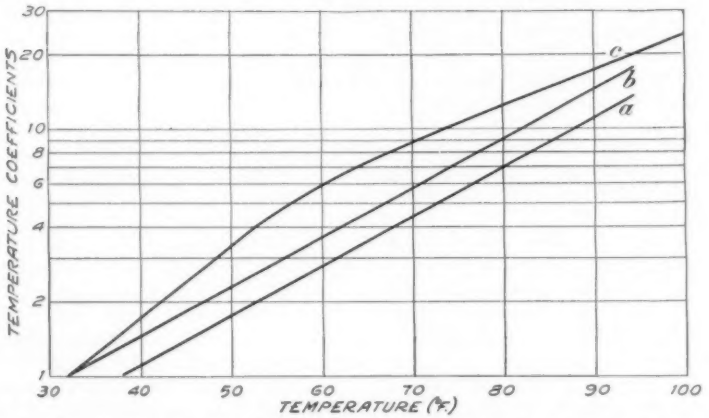

FIGURE 3.—Preliminary and final temperature coefficients for spinach as determined by a method of approximations. See text for explanation.

corresponding coefficients on a logarithmic scale (fig. 3, *a*). Assuming that the decrement between 36° and 32° continues at the same rate, a second line, *b*, could be drawn parallel to line *a* in such a way that line *b* intersects the base line at 32°. Thus it was possible to obtain temperature coefficients the numerical values of which were based on a coefficient of 1 for 32°, and it was also possible to obtain values for the constant K which would show in comparable numerical terms how much longer one kind of vegetable can be held than another at that temperature.

Next it was necessary to eliminate the error which was introduced when the arithmetic rather than the logarithmic mean of the temperature curves (fig. 1) was used in calculating the coefficients. This was done by determining separately for each 5-hour interval the average temperature and the corresponding approximate coefficient, using the values given by curve *b*, figure 3. Next, each coefficient was multiplied by 5 and the sum of these products was calculated separately for each temperature curve, thus giving the values for $C_t \times h$ of each experimental lot. Provided the true values for C_t had been

found, the product $C_t \times h$ should be the same, and should equal a constant K for every lot of the same vegetable regardless of the temperature at which it was stored. Table 1 shows the values for K as calculated from the temperature coefficients represented by figure 3, *b*. While the calculated values of K for the lots stored at 32° and 80° agree fairly well they are much too low for those kept at 50° and 65°. In calculating temperature coefficients for other vegetables by the same method, it became apparent that the coefficients for the temperature range between 50° and 70° F. always gave too low values. This led to the conclusion that the increase in the rate of deterioration of vegetables is not uniformly logarithmic over the entire temperature range used in these experiments. Instead, when plotted on a semi-logarithmic scale as in figure 3, the true rate curve consists of two separate, fairly straight lines which intersect somewhere between 50° and 60°. Based on this observation, a third curve, *c*, was drawn, the slope of which was steeper at the lower and flatter at the higher temperatures. The exact position of this curve was determined by a series of approximations. The slope of the two sections was changed until finally values for the temperature coefficients were obtained, which, in calculating the K values of the four experimental lots, gave results which were practically identical (table 1). These final values for K show less than 1-percent deviation from the mean, indicating that the temperature coefficients as represented by curve *c* (fig. 3) correspond accurately to the true rate of deterioration at the different temperatures.

It should be pointed out that while this method of calculating the temperature coefficients is indirect and one of trial and error, the final results obtained are nevertheless very accurate and the degree of error is expressed exactly by the deviations from the mean of the K values as calculated for different experimental lots. While the temperature coefficients were calculated from the experimental data of four different lots only, the results are actually based on more than 50 single observations, since the average temperature for each 5-hour interval was considered in determining the exact position of the coefficient curves. The only assumptions made are that the coefficient when plotted against the temperature must follow a single smooth curve; also that the rate decrement between 36° and 32° F. is about the same as in the range between 50° and 36°.

The coefficients of the other vegetables were calculated in the same manner. The process of arriving at the true values was repeated in every case until the deviations from the mean value of K was never greater than 3 percent.

This method of arriving at the temperature coefficients may be criticized as being unnecessarily cumbersome and the suggestion may be offered that the same results could have been obtained more easily by making provision for bringing the vegetables to a constant temperature very rapidly, thus avoiding the complications due to temperature fluctuations in the experimental lots. The only way this could be accomplished is by spreading the vegetables on the floor of the constant-temperature room. Preliminary experiments showed that by doing so a constant temperature was attained within a few hours and subsequent reheating of the vegetables was prevented effectively. On the other hand, it was noticed that these vegetables when spread

out were subject to excessive wilting. It was also found that the rate of deterioration and the symptoms of break-down under these conditions were somewhat different from those observed in lots stored in commercial containers. On the basis of these findings, it was felt that the method of experimentation actually used would yield data which are more useful in solving such practical problems as are met in connection with precooling, transportation, and temporary cold storage of vegetables.

RESULTS AND DISCUSSION

TEMPERATURE COEFFICIENTS

The temperature coefficients of seven different vegetables showing their relative rate of deterioration are given in figure 4. The temper-

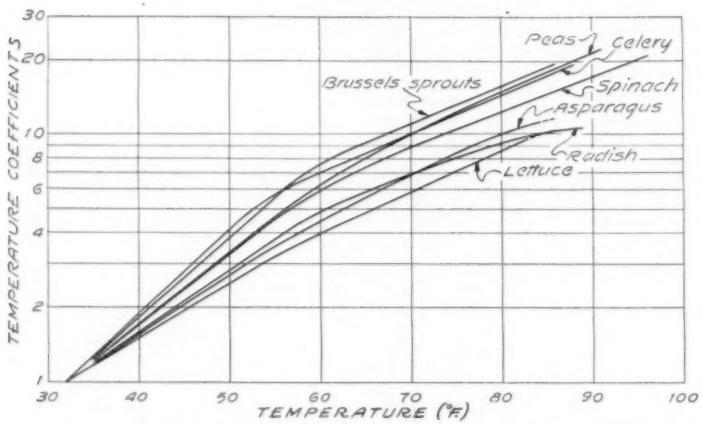

FIGURE 4.—Coefficients indicating the rate of deterioration of seven vegetables at different temperatures. Values are based on a coefficient of 1 for 32° F.

ature of the vegetable is plotted on the X axis on an algebraic scale, while the corresponding coefficients are plotted on the Y axis on a logarithmic scale. Each resulting curve follows two fairly straight lines which intersect between 50° and 65° F. In other words, the increment in the rate of deterioration is fairly uniform below 50° and again above 65°, the increment being more rapid in the lower than in the higher temperature range. The break in each one of the curves can be explained only by assuming that above 50° a new, important factor enters the biological reactions that determine the rate of break-down. Most likely this new factor is closely associated with the respiration occurring in the plant tissue. Other factors which may be responsible for the observed change in the reaction rate are the activity of various enzymes and the growth rate of micro-organisms at different temperatures. Many experiments have shown that below 77° the respiration rate of plant tissue increases logarithmically with a rise in temperature. Above that temperature the rate continues to increase, but much more slowly, until finally a point is reached beyond which the respiration rate declines rapidly to a very

low level. Thus Gerhart (*5*), studying the rate of respiration in strawberries, found that above 77° the increment in the respiration rate was less than below that temperature. Similarly, Gore (*6*) found that the increase in the respiration rate of various fruits was slightly reduced at higher temperatures. However, in both these studies the break in the respiration curve did not become very pronounced until a temperature of 90° was attained. Gerhart explains the break in the respiration curve by assuming that at high temperatures the permeability of the cell wall to gases becomes a limiting factor, causing a deficiency of oxygen and an accumulation of carbon dioxide and other end products in the plant tissue. Whether or not changes in the respiration rate are the principal cause for the observed break in the deterioration curves of vegetables remains questionable. Doubt may be cast on such an explanation in view of the fact that the break in these curves occurred at temperatures between 50° and 65°, while Gerhart, Gore, and others found that the respiration rate of fruits increased logarithmically until the temperature reached 77° or 95°. It must be kept in mind, however, that in the last-mentioned studies on respiration only a few isolated fruits were used. It is quite possible that even at a temperature of 55° the oxygen concentration within a commercial container becomes sufficiently low to cause an appreciable lowering of the normal respiration rate. This reduction in the respiration rate would not result under conditions where relatively little plant tissue is in contact with air of normal atmospheric composition as was the case where the respiration rate of fruits and vegetables was being studied experimentally.

It is interesting to compare the temperature coefficients of different vegetables for any one temperature. One might suspect that those vegetables that are usually considered as being highly perishable have also a high temperature coefficient. An inspection of figure 4, however, shows that such is not the case. On the contrary, brussels sprouts, peas, and celery, which resist deterioration in storage for a relatively long period of time, had the highest coefficients. In other words, a definite increase or decrease in the storage temperature affected the total storage life of these vegetables more than it did that of asparagus, radish, and lettuce, which are subject to comparatively rapid break-down even at 32°. While this unexpected relationship holds true fairly well in the seven vegetables studied, it would be necessary to carry out experiments with a large number of other vegetables to find out whether or not these observations can be stated as a general rule. It should be emphasized here that the temperature coefficients are not a measure of the actual rate of deterioration at any one temperature. Instead, they merely indicate the degree of acceleration of the deterioration process in comparison with the rate at which this process proceeds in the same vegetable at 32°. Celery, for instance, although it had relatively high temperature coefficients, never attained the same rate of break-down as some of the other vegetables even in the higher temperature range, simply because the basic rate of break-down at 32° was very much lower than it was in the more perishable vegetables.

RATE OF DETERIORATION AND VAN'T HOFF'S RULE

It has frequently been proved that most chemical and biochemical reactions in vitro follow Van't Hoff's rule fairly closely. This rule

states that the reaction rate increases two or three times with every 10° C. rise in temperature. It has also been shown that many physiological processes of micro-organisms and higher plants, such as growth and respiration, conform at least roughly to Van't Hoff's rule. Based on these observations this rule has been cited to illustrate the important relationship between temperature and the rate of deterioration of fruits and vegetables. Thus Kidd and West (7) showed that both the rate of break-down and the rate of respiration of apples in storage obey Van't Hoff's rule at temperatures ranging from −1° to +18°. The rate factor indicating exactly how much the reaction rate increases with a 10° rise in temperature is usually expressed as Q_{10}.

From the temperature coefficients given in figure 4 it was easy to calculate the Q_{10} values of the seven vegetables at three temperature ranges of 10° C. each (table 2).

TABLE 2.—*Q_{10} values for the 7 vegetables considered in Figure 4 at 3 different temperature ranges*

Temperature range (°F.)	Celery	Brussels sprouts	Peas	Spinach	Radish	Lettuce	Asparagus
32–50	4.12	3.82	3.33	3.33	2.87	2.48	2.68
50–68	2.27	2.70	2.76	2.49	2.26	2.22	2.37
68–86	1.94	1.87	2.03	1.83	1.58	1.87	1.84

First of all it will be observed that at the lowest temperature range of 32°–50° F. celery and brussels sprouts have the highest Q_{10} values. As the temperature is raised, the Q_{10} values decrease in all vegetables, more rapidly in those which at the lower temperature had relatively high-values, so that at the highest temperature range of 68°–86° the Q_{10} values became fairly uniform for all vegetables, varying only from 1.58 to 2.03. The important conclusion derived from table 2 is the fact that the Q_{10} value expressing the increment in the rate of deterioration follows Van't Hoff's rule very roughly only. The calculated values not only vary with different vegetables, but they also change with different temperature ranges for the same vegetable. It is not permissible therefore to apply these rate values to temperature ranges other than those for which they were found experimentally.

TIME-TEMPERATURE CURVES

From the data presented in figure 4, it was possible to express the temperature relationship in the form of time-temperature curves (fig. 5) similar to those presented in figure 2. In both cases the data are plotted on the same scale; the only difference is that in figure 2 the maximum storage life of the vegetables is plotted against the temperature of the storage room, while in figure 5 it is plotted against the temperature of the vegetable itself. Consequently figure 2 is more useful in determining the possible storage life of vegetables held at a constant temperature, while the data presented in figure 5 should be helpful in studies where records are available of the temperature of the vegetables regardless of whether these temperatures remain constant or fluctuate over a wide range.

Regardless of the method used for calculating the time-temperature curves, the data show that a lowering of the temperature by a definite

number of degrees in the range above 55° F. has very little effect on the total storage life of the vegetables studied, while the same decrease in temperature below 55° causes a very appreciable lengthening of their life span. This, of course, is due to the fact that the Q_{10} values are much smaller in the upper than in the lower temperature range. These findings are contrary to the common belief that the increment in the deterioration rate becomes progressively greater as the temperature is raised.

Applying these findings to practical problems of storage and transportation, one must conclude that any precooling, icing, or refrigeration method used is of little benefit unless it is sufficiently effective to lower the temperature of the vegetables to 55° F. or lower. In other words, the effect of refrigeration in delaying ultimate break-down

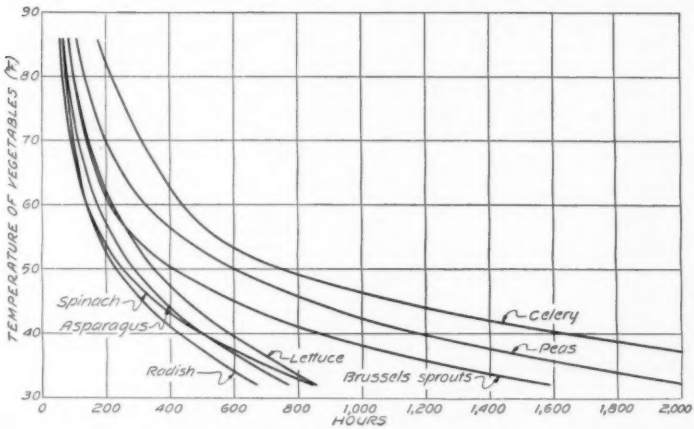

FIGURE 5.—Time-temperature curves showing the time required to bring about complete deterioration of seven different vegetables if the temperature of the vegetables is the same as indicated on the ordinate.

becomes increasingly greater as the lower temperature range is approached. A comparison of figures 2 and 5 shows the total storage life of the different vegetables at any one temperature to be somewhat longer when the data were based on the temperature of the vegetables rather than on the temperature of the room. This is to be expected because the actual average temperature of the vegetables is always higher than that of the storage rooms.

THE APPLICATION OF TEMPERATURE COEFFICIENTS AND TIME-TEMPERATURE CURVES TO PRACTICAL PROBLEMS

The data presented in figures 3 and 5 not only make it possible to predict how long a certain vegetable will keep, provided its temperature history is known, but they also permit the calculation of the relative effectiveness of different shipping or precooling methods. The procedure to be followed is similar to that used in calculating the temperature coefficients. First, it is necessary to find the temperature coefficients corresponding to the average temperature of

small intervals on the temperature curves. Next, each coefficient is multiplied by the respective number of hours of the time interval and the sum of these products is determined. The fraction that this sum is of the constant K of that particular vegetable will indicate exactly how far deterioration of the lot in question has proceeded. In this way it can be shown, for instance, that a lot of peas properly precooled and shipped from the West coast to New York City is likely to arrive in better condition than a similar lot which is shipped by truck from within New York State without precooling. Using hypothetical data, it was calculated that deterioration on arrival had advanced to the extent of 20 percent in the first and 35 percent in the second lot. This difference in conditions in favor of the peas from the West coast prevails in spite of the fact that this lot was in transit for 10 days, while the peas from New York State were assumed to have reached the market within 30 hours.

Here again it should be pointed out that any differences in the degree of deterioration as calculated from the temperature coefficients are not necessarily reflected in their external appearance. When an attempt was made to measure the rate of deterioration of different vegetables by means of a grading system, it was found that the first symptoms of break-down could not be recognized easily until one-fourth or one-third of the total deterioration process was completed. Also, it was noticed that the rate at which visible changes in appearance took place at any one temperature became progressively more rapid as the final stages of break-down were approached. In other words, the rate of visible break-down under definite conditions is not all uniform throughout the entire process. Consequently, the temperature coefficients apply strictly speaking only to the rate of the complete process; they cannot be used safely to determine how far visible deterioration may progress under certain conditions within a given time interval.

Needless to say, any calculations based on figures 1 to 5 can give results which are only approximately correct. While the condition of the vegetables used in these experiments was probably fairly representative of good commercial lots, there are many variables such as variety, growing conditions, and stage of maturity that may have a pronounced effect on the keeping quality of a certain vegetable. It seems reasonable to assume that although these variables may change the constant K to an appreciable extent, there is less likelihood that they will have much influence on the temperature constant of any one kind of vegetable.

EFFECT OF PREVIOUS COLD STORAGE ON THE RATE OF DETERIORATION AT HIGHER TEMPERATURES

In recent studies on the respiration of vegetables under various conditions, Appleman and Smith (*2*) found that the respiration rate of certain kinds is temporarily accelerated as a result of previous prolonged exposure to low temperatures. These findings would suggest that precooling or temporary cold storage may have a detrimental effect on the keeping quality of vegetables at subsequently higher temperatures. Such an assumption is in agreement with the belief held by many growers and shippers that vegetables after precooling tend to deteriorate more rapidly than other lots which have not been

precooled. Obviously, if the contention were correct that the previous temperature history of a vegetable has a direct effect on the subsequent rate of break-down the results of this investigation would be of doubtful value, except in those cases where the temperature remained fairly constant throughout the storage period.

A series of experiments was carried out by the writer in which several vegetables were held at 35° F. for periods ranging from 1 day to several weeks and then were transferred to higher temperatures. The results of these studies will be described in a separate paper. It suffices to say that in no case did the initial low temperature have any noticeable effect on the rate of break-down at higher temperatures. In general, where the vegetables had been held at 35° for more than 1 week, they did not keep as long at subsequently higher temperatures as those which had not received the low-temperature treatment. However, this should be expected because even at 35° deterioration takes place, although the rate is very slow. Taking into account the temperature history of each lot of vegetables in its entirety, the product of time and the corresponding temperature coefficients gave results very nearly alike, which is in full agreement with the conclusions derived from the present study. It is safe to say, therefore, that the efficiency of any precooling, handling, and transportation method can be determined with a reasonable degree of accuracy by the method outlined, provided the temperature history of the lot and the temperature constants of the particular vegetable are known.

RATE OF SUGAR LOSSES IN ASPARAGUS, SWEET CORN, AND PEAS AT DIFFERENT TEMPERATURES

In calculating the temperature coefficients given in figure 4, the external appearance of the vegetables was used exclusively as a basis for determining the point at which they reached the stage of complete break-down. It was pointed out, however, that the rate at which visible deterioration takes place is not necessarily the same as the rate at which the edibility of a vegetable changes after harvest. In fact, some preliminary experiments suggested that at least in some vegetables a fairly wide discrepancy exists between these two rates. It was for this reason that a second series of temperature coefficients was calculated for asparagus, sweet corn, and peas. These new coefficients were based on the rate at which sugar disappeared from the stored lots.

The rate at which the percentage of total sugars decreased in three different vegetables when held at 35°, 50°, 65°, and 80° F. is expressed in figure 6. It will be noticed that the rate of sugar depletion is by no means uniform. In some cases, notably in sweet corn (*B*), the rate at which the percentage of sugar decreases becomes much slower as the supply diminishes. Under certain conditions there may be an actual increase in the percentage of sugar present. Obviously the quantity of sugar present at any one time depends on the rate at which sugar is oxidized in the process of respiration and at the same time is replenished by the conversion of starch to sugar. These two processes may have different temperature coefficients and both are controlled by the law of mass action.

The calculation of the temperature coefficients was based on the time interval which causes the sugar content to decrease to 70 percent of its original value at any one temperature. The level of 70 percent was chosen arbitrarily because during this period the rate of sugar

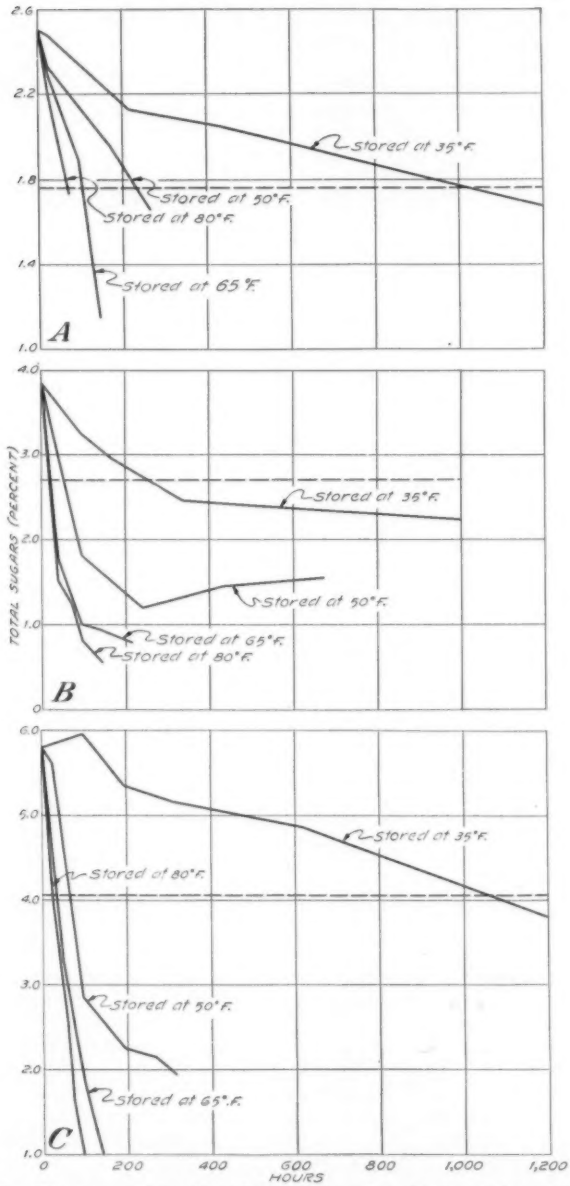

FIGURE 6.—The percentage of total sugars, based on fresh weight, of four lots of asparagus (*A*), sweet corn (*B*), and peas (*C*) when stored at 35°, 50°, 65°, and 80° F. for different periods. In each instance the broken horizontal line indicates the stage at which 30 percent of the original sugar content had disappeared.

loss remains fairly uniform, and also because actual tasting showed that a 30-percent loss of sugar is associated with a very noticeable decrease in sweetness.

Using the method described earlier, the calculations of the temperature coefficients were based on the temperature of the vegetables in storage rather than on the temperature of the storage rooms. The results of these calculations are given in figure 7. As compared with the data given in figure 4 they show that the temperature coefficients for asparagus and peas are more than twice as high when calculated

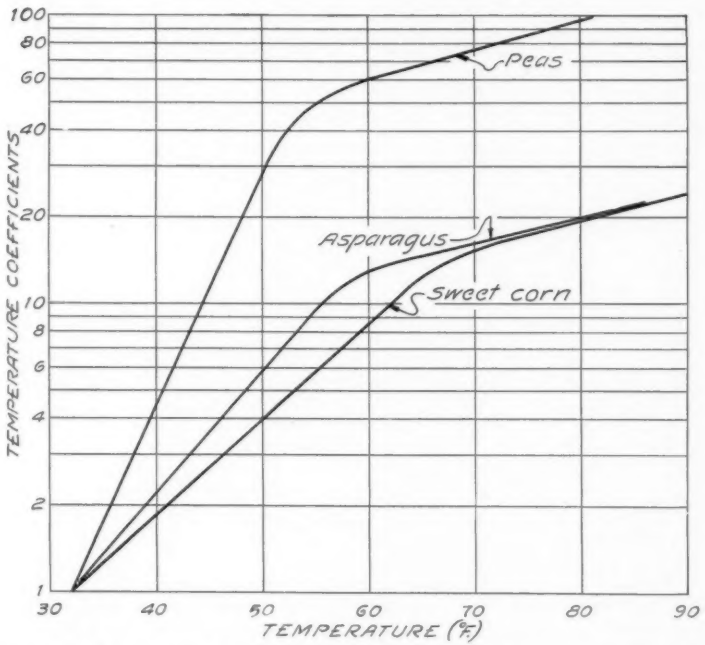

FIGURE 7.—Coefficients indicating the rate at which 30 percent of the original sugar content disappears in asparagus, sweet corn, and peas at different temperatures.

on the basis of sugar depletion as when determined by the rate at which visual deterioration occurs.

The Q_{10} values for the different temperature ranges are given in table 3.

TABLE 3.—*Q_{10} values for 3 vegetables based on their rate of sugar depletion at 3 different temperature ranges*

Temperature range (° F.)	Peas	Asparagus	Sweet corn
32–50	27.5	5.8	3.9
50–68	2.6	2.7	3.6
68–86	1.5	1.4	1.5

An exceedingly high Q_{10} value was found for peas at the lowest temperature range. The explanation for this is the fact that at temperatures close to the freezing point sugar depletion of peas as a result of respiration is very slow and is almost balanced by the rate at which starch is converted into sugar. Comparing the Q_{10} values at the temperature range of 68°–86° F. in tables 2 and 3, it will be noticed that there is little difference in the rate increment whether deterioration is measured by visual changes or by chemical analysis. Attention should also be called to the fact that the break in the rate curve occurs at about the same temperature in both cases (figs. 4 and 7).

Appleman and Arthur (*1*) calculated Q_{10} values for sweet corn by a similar method. In general their calculated values are lower than those found in the experiments carried out by the writer. This discrepancy may be accounted for in part by the fact that Appleman and Arthur were dealing with single ears of corn, while the writer

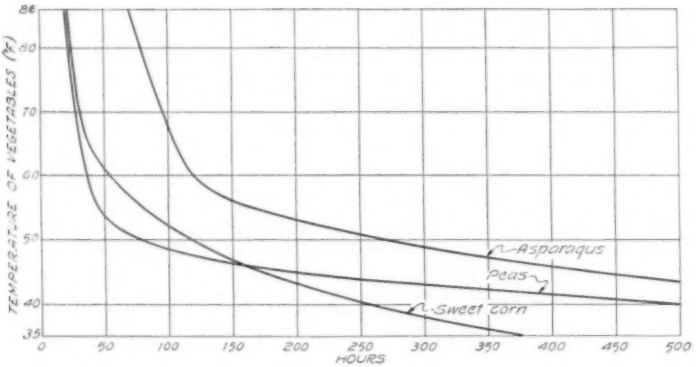

FIGURE 8.—Time-temperature curves showing the time required to produce a 30 percent loss of the original total sugar content of asparagus, sweet corn, and peas when the temperature of the vegetables is that indicated on the ordinate.

obtained his samples from commercial packages. It remains difficult to explain, however, why the Q_{10} values obtained by Appleman and Arthur for the range of 50°–68° F. were consistently lower than they were for the temperature ranges above and below this interval.

In order to show the time required to bring about a 30-percent decrease in the sugar content at any given temperature the data were expressed in the form of time-temperature curves (fig. 8) similar to those presented in figure 5. It is of practical significance that at all temperatures the rate of sugar losses in sweet corn and peas is more than twice as rapid as in asparagus. Furthermore, it should be pointed out that at high temperatures, peas and sweet corn lose their sugar at nearly the same rate, while at low temperatures the sugar losses are retarded much more in peas than in sweet corn. Comparing the time-temperature curves in figures 5 and 8 for the same vegetable, it becomes apparent that at any given temperature the time required to bring about a 30-percent decrease in the sugar content of asparagus is roughly the same as the period necessary for complete deterioration, as measured by external appearance. On the other hand, peas at an

average temperature of 85° F. lose 30 percent of their original sugar content in less than 20 hours, whereas they do not become unmarketable on account of their external appearance until the end of 110 hours. In other vegetables the discrepancy between the rate of sugar depletion and that of visible deterioration may be even more pronounced. Parker and Stuart (*8*), for instance, have shown that even at room temperature the percentage of sugar in snap beans actually increases slightly throughout the storage period as a result of a rapid rate of hydrolysis of starch to sugar.

SUMMARY

Seven different vegetables were held in storage at 35°, 50°, 65°, and 80° F. Accurate records were taken of the temperature of the vegetables themselves and of the time required to cause complete deterioration of the different lots.

When stored in commercial containers, the temperature of the vegetables may vary widely from the temperature of the room in which they are stored.

Based on the experimental data, a series of temperature coefficients, Q_{10} values, and time-temperature curves were calculated to show the integrated rate at which these vegetables deteriorate at different temperatures and also to show the rate of acceleration of this process when the temperature is raised by a definite number of degrees.

It was found that the temperature coefficients, when plotted against temperature on a semilogarithmic scale, give two fairly straight lines which intersect between 50° and 65° F. The rate of increment in deterioration as a result of rising temperatures was always higher in the lower than in the upper temperature range. Corresponding differences were found in the Q_{10} values, which varied from 2.48 to 4.12 in the temperature range of 32° to 50° with a corresponding variation of 1.58 to 2.03 at temperatures between 68° and 86°.

A comparison of the Q_{10} values in the lowest temperature range showed that in the seven vegetables studied, the Q_{10} values were appreciably lower for those vegetables that usually are considered as being highly perishable than for celery and brussels sprouts which are regarded as less perishable. This suggests that cold storage increases the life of easily perishable vegetables not nearly so much as it does that of the less perishable ones.

The findings emphasize the fact that the Q_{10} values for the rate of deterioration vary not only with different vegetables but apply only to the temperature range for which they were calculated from experimental data.

The time-temperature curves show that the period for which vegetables can be held in storage is lengthened only very slightly when the temperature is lowered from 80° or 90° to 55° F. On the other hand, the life span may be increased very decidedly by a corresponding lowering of the temperature below 55°. Any precooling method, therefore, is of little use unless cooling to temperatures below 55°. can be accomplished.

Temperature coefficients and time-temperature curves may be used to predict the length of total storage life of a vegetable. They also furnish a basis for calculating the effectiveness of different pre-

cooling or transportation methods, provided records are available to show the temperature changes occurring in the vegetables themselves.

Holding vegetables at a temperature of 35° F. from a few days to several weeks had no noticeable effect on the subsequent rate of break-down when these vegetables were later transferred to higher temperatures.

The rate of visible break-down and the rate of deterioration in eating quality are not necessarily the same. The Q_{10} values of asparagus, sweet corn, and peas differed widely, depending on whether the rate of deterioration was measured by visible break-down or by the rate at which sugar depletion occurred in these vegetables. A Q_{10} value of 27.5 was calculated for the increment in the rate at which peas lose 30 percent of their original sugar content in the temperature range between 32° and 50° F. On the other hand, the Q_{10} values for this same process were found to be very low in the upper temperature range, varying from 1.4 to 1.5 in the three vegetables studied.

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DIFFERENTIAL SURVIVAL OF ALFALFA STRAINS UNDER AN ICE SHEET[1]

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INTRODUCTION

Winter injury of alfalfa (*Medicago sativa* L.) appears principally in three forms in the Great Lakes region, namely, heaving, direct damage from low temperatures, and "smothering" under ice sheets. Data are not available on the relative destructiveness of these three agencies. Heaving is common and may be an important cause of impairment of stands. Willard, Thatcher, and Cutler (*10*)[2] attribute most of the winter killing of alfalfa in Ohio to it. Considerable injury from low temperatures in the absence of ice sheets likewise occurs. The most devastating losses, however, in southern Wisconsin at least, result from ice sheets. When these form directly on the soil surface, that is, without an intervening layer of snow, they are apt to be especially destructive. Bacterial wilt, caused by *Phytomonas insidiosa* (L. McC.) Bergey et al., has made serious inroads in parts of this region during the last decade and adds to the complexity of the winter-injury problem. Jones (*7*) has pointed out that, when sources of infection are about the same, the disease is likely to be much worse in injured than in uninjured fields.

A major part of the change in acres of alfalfa cut for hay in Wisconsin between 1936 and 1937 is attributable to an ice sheet which occurred during the intervening winter. In an area comprising 21 southeastern counties there was a net loss of about 250,000 acres, or 43 percent. The change in this direction reached its maximum in Jefferson and Waukesha Counties, where 80 percent of the alfalfa was destroyed.

The ice sheet formed January 6 to 8, 1937, when 1.54 inches of rain fell at Madison at air and soil temperatures which resulted in its freezing as it reached the surface. The duration of the ice sheet varied somewhat depending on locality and exposure, but it was not until February 13 that level areas were free around Madison. The countryside was encased in heavy ice, therefore, for about 5 weeks. The storm affected all the southern two-thirds of Wisconsin except the western counties near the Mississippi and St. Croix Rivers, where ice was not formed. When it became evident in April that alfalfa had been severely damaged by the ice, plans were made to measure the amount of injury in 42 strains in a replicated planting in the breeding nursery. Although the plot had been laid out with another purpose in view, it proved to be well suited for a study of this problem.

[1] Received for publication November 10, 1938. Paper No. 232 of the Department of Genetics, Wisconsin Agricultural Experiment Station.

[2] Italic numbers in parentheses refer to Literature Cited, p. 71.

THE IRREGULAR DISTRIBUTION OF ICE-SHEET INJURY

Ice-sheet injury tends strongly to occur in patches. This fact is well illustrated by the condition of the experimental plot in question. The approximate relative position of each surviving individual was recorded on quadrillé paper in May 1937. The distribution of the survivors over the area is shown in figure 1. The position of each

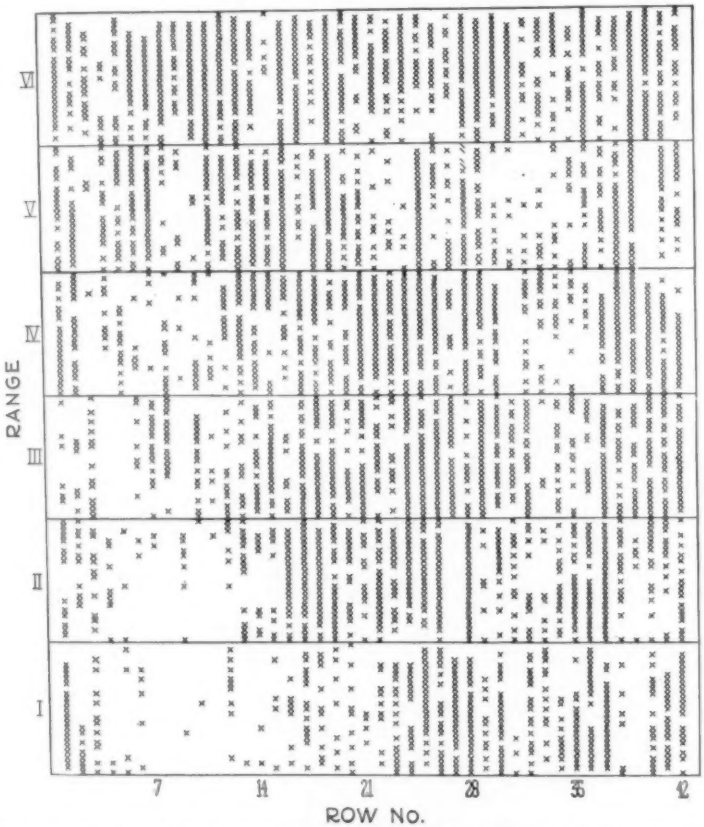

FIGURE 1.—Distribution of surviving alfalfa plants in the spring of 1937 on the experimental plot at Madison, Wis., following ice-sheet injury.

strain in each replication is given in table 1. Although the different strains are scattered on the plot at random, with certain restrictions to be pointed out later, it is readily apparent from the diagram that, regardless of strain, many more plants were killed in some areas than in others nearby. It might be inferred from the patchy character of ice-sheet injury that differences in the hereditary constitution of individual strains or plants are unimportant in determining survival. Indeed, the general appearance of damaged commercial fields, and the fact that, rather commonly, varietal trials afford no positive

evidence of such a relation, might easily lead one to conclude that genetic differences in resistance to ice sheets are entirely wanting. The question, however, is not so easily dismissed. Field observations under farm conditions can rarely be expected to meet the requirements of the case, and it is difficult to obtain competent evidence even from an experimental trial. The chief reason for the latter is the lack of control over the ice-sheet variable.

TABLE 1.—*Planting arrangement of the alfalfa strains replicated in the experimental plot at Madison, Wis.*[1]

Range	Alfalfa strain Nos. planted in various rows of—																			
	Column I							Column II							Column III					
VI	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
V	22	31	26	34	24	40	29	17	36	28	39	30	25	37	23	2	27	41	7	35	5
IV	11	39	27	21	23	38	23	5	35	41	26	19	33	29	42	14	36	10	34	6	13
III	42	14	36	28	18	25	33	6	15	34	27	3	32	40	1	38	30	39	8	12	31
II	13	19	9	30	16	35	10	1	22	18	38	23	20	4	28	33	24	29	40	26	37
I	8	17	37	12	41	20	15	42	24	16	2	31	21	7	11	3	32	25	9	22	4

Range	Alfalfa strain Nos. planted in various rows of—																			
	Column IV							Column V							Column VI					
VI	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42
V	42	38	14	8	12	4	13	1	21	9	18	16	10	6	32	19	33	11	15	3	20
IV	9	3	31	37	30	18	40	4	8	15	20	22	7	12	28	1	17	2	24	16	25
III	2	21	16	20	7	19	35	11	24	17	41	23	13	37	5	26	9	22	4	10	29
II	11	34	6	17	41	15	32	42	25	2	39	5	3	36	8	31	14	27	7	21	12
I	1	39	33	29	10	36	5	27	40	38	19	28	14	26	6	30	23	18	34	13	35

[1] The strains were replicated 6 times in rows 12.5 feet long and 24 inches apart, the plants being spaced at approximately 6 inches in the row. Each strain occurred once in each of the 6 ranges (running north and south) and once in each of the columns. Each column was 7 rows wide.

Ice-sheet injury apparently is a complex phenomenon. There are formidable obstacles in the way of a planned test for genetic differences between strains. We are virtually dependent at present upon such evidence as is afforded by the occasional field trial which happens to encounter the conditions under which genetic differences, if present, may appear. What these conditions are cannot be fully specified. Two requirements, however, may be stated: (1) The average level of injury must be such that a minimum number of strains fall in the extreme classes of 100-percent killing or 100-percent survival; (2) in order that an informative analysis of the results be made, the experiment must be so designed as to permit the segregation of possible hereditary differences from other, frequently large, sources of variability. As the level of injury in the field is beyond control, and the time, place of occurrence, and duration of ice sheets cannot be readily predicted, there is a considerable element of chance in obtaining significant data on the problem. That such evidence is available in the present study is due to the circumstance that the main requirements, as laid down above, happen to have been met.

Assuming that alfalfa strains vary in resistance to ice sheets, over how wide a range of test conditions is a difference maintained? General observations indicate that differences in survival between genotypes disappear as the severity of an ice sheet reaches a certain level. In commercial fields, for example, sown to one of the ordinary varie-

ties, which comprises a diverse assemblage of genotypes, there may be a sharp line of demarcation between areas in which, on the one hand, all the plants are killed and, on the other, most of them live. In other words, the magnitudes of the differences in survival between strains are a function of the severity of the conditions, and above a certain point, hereditary variations, which may be significant at lower levels, are completely overridden. This relationship must be considered one of the most important aspects of the problem.

The present experimental data are based on a single trial in which the plants were exposed rather uniformly to a particular set of ice-sheet conditions. Were these conditions severe, moderate, or mild? If the relation postulated in the preceding paragraph holds, it is necessary to answer this question in order that the findings be understood in their bearing on the general problem of ice-sheet injury.

THE RELATIVE SEVERITY OF THE ICE ON THE EXPERIMENTAL PLOT

The damage done to alfalfa by the 1937 ice sheet varied greatly over the extensive area within which the experimental plot lay. It is possible to establish with fair accuracy the amount of damage done by the ice at Madison where the experimental plot was located, as compared with that in the surrounding sections, from the assessor's reports [3] of acres of alfalfa cut for hay in 1936 and 1937. The net change during this interval is shown for Wisconsin by counties in figure 2. Each black dot represents a net loss of 200 acres and each hollow circle a net gain of the same amount. Furthermore, from the replies to a questionnaire sent by the Federal-State Crop Reporting Service for Wisconsin to local reporters in February 1937 concerning ice-sheet conditions on their respective farms, the position of the plot is revealed relative to an area in which the destructive effects of the ice were more or less offset by an important protective factor, namely, a sub-layer of snow.

South of a straight line drawn in figure 2 from the southeastern corner of Door County to the southwestern corner of the State the soil tended to be free from snow at the time the ice sheet developed; north of this line a more or less continuous covering of snow was present on top of which the ice formed as a crust. More accurately speaking, the line passes through a transition zone between an area uniformly covered with snow to the north and a snow-free area to the south.

One is immediately impressed with the relationship between the line dividing the region in which losses occurred from that in which gains are shown and the boundary of the snow cover at the time the ice sheet was formed. The two lines coincide, so far as the available records permit of their positions being determined. The ice was extremely destructive where it formed directly on the soil and much less injurious where there was snow underneath.

The experimental plot at Madison was located in the transition zone between the area bare of snow and that covered with snow when the ice formed. This is clearly brought out in figure 3 in which the net changes in acreage harvested for hay in 1936 and 1937 for Dane

[3] The writers are greatly indebted to Dr. W. H. Ebling, senior agricultural statistician, Federal-State Crop Reporting Service for Wisconsin, for supplying these data in advance of publication. He has likewise made available for the writers' use the replies to the questionnaire on ice-sheet injury.

County are plotted by townships. The mortality on the plot was high, as will be seen later, but the level of injury was below the point which led to significant acreage decline elsewhere in the State. An appreciation of this fact is important in interpreting the data obtained.

ORIGIN OF THE STRAINS

The 42 strains whose reaction to the ice sheet was studied formed a relatively homogeneous group. Two checks were included, one of which was Grimm, a well-known hardy variety widely grown in the

FIGURE 2.—Net change in acres of alfalfa cut for hay between 1936 and 1937 in Wisconsin by counties. Each dot represents a net loss of 200 acres over 1936, and each hollow circle a net gain of equal amount. (Based on assessor's reports to Dr. W. H. Ebling, senior agricultural statistician, Federal-State Crop Reporting Service for Wisconsin.)

State, and the other, Hardigan, which is similar in type and cold resistance. The remaining strains, for the most part, were either single plant lines (once, twice, or three times self-pollinated) selected from the Grimm variety on the basis of cold resistance, as measured by an artificial freezing test, or single, three-way and double hybrids involving these strains. One strain similarly selected from the Cossack variety was also represented, both as a single plant line and in a few

of the hybrids. A five-times self-pollinated line derived from Turkestan alfalfa was the only stock included which was resistant to bacterial wilt. In a rather drastic test involving artificial inoculation with the bacterium this strain had previously been found to contain approximately 50 percent of highly wilt-resistant individuals. All the seed used in the plantings, except that of the check varieties, was produced in the greenhouse by controlled hand-pollination.

The relative uniformity of origin of the strains under test is important in gaging the significance of the results. Obviously, it means

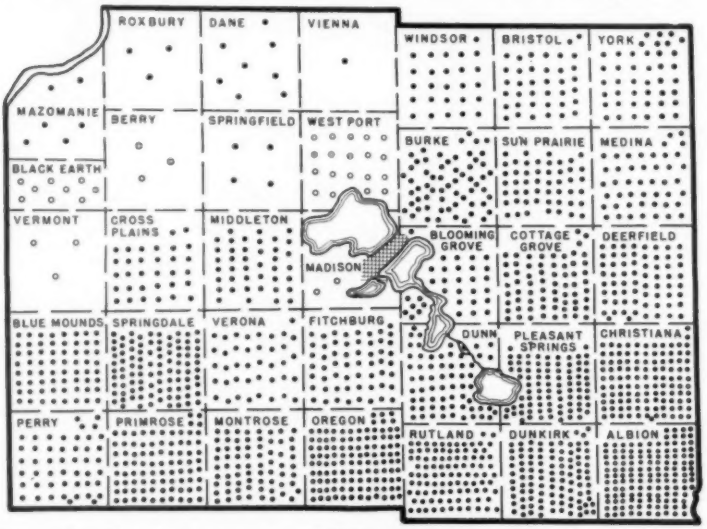

FIGURE 3.—Net change in acres of alfalfa cut for hay between 1936 and 1937 in Dane County, Wis., by townships. Each dot represents a net loss of 10 acres over 1936, and each hollow circle a net gain of equal amount. (Based on assessor's reports to Dr. W. H. Ebling, senior agricultural statistician, Federal-State Crop Reporting Service for Wisconsin.)

much more to the breeder if differences in resistance to ice-sheet injury exist in strains derived from varieties already rather well adapted to the region than if such differences can only be demonstrated in more widely diverse stocks.

THE EXPERIMENTAL PLOT

The plot was laid out in the form of a modified Latin square. The strains were replicated six times in rows 12.5 feet long and 24 inches apart, the plants being spaced at approximately 6 inches in the row. Each strain occurred once in each of six ranges, running north and south, and once in each column, seven rows wide, running at right angles. Except for these restrictions, the strains were grown in random order as shown in table 1.[4]

[4] This type of arrangement was suggested by Student (*9*) and is frequently referred to as the "semi-Latin square." Although this arrangement provides efficient, unbiased estimates of the true percentage mortalities for the 42 strains, Yates (*11*) has pointed out that designs of this type, when analyzed according to the usual analysis of variance procedure appropriate to a Latin square, suffer from a biased error mean square, and Goulden (*6*) has evaluated an expression for this bias. The probable nature of the bias in the present experiment and its effect on the inferences drawn are discussed later (p. 67).

An even slope, nearly parallel to one diagonal, gave the plot a relatively uniform relief. The fall along this diagonal was 2.3 feet in approximately 115 feet. Excellent surface drainage was thus provided. Only in one corner, where a slight depression crossed the plot, did moisture conditions appear to change significantly. Mortality in this area was especially high.

The soil was fertile Miami silt loam, in good tilth. It had been limed a few years earlier to correct a slight acidity. The natural underdrainage was good.

The seed was sown by hand in early summer, 1935, and, with a small amount of replanting, an excellent stand was obtained. One cutting, bearing a crop of seed, was taken in July 1936. The summer was extremely hot and dry, and water being available, the plot was irrigated once after the seed pods had formed. Fall rains were abundant, however, and the plants entered the winter of 1936–37 bearing a large growth of shoots.

BACTERIAL WILT ON THE EXPERIMENTAL PLOT

The soil in this area is known to be infested with *Phytomonas insidiosa*, the causal organism of bacterial wilt of alfalfa. The extent to which the disease contributed to the killing of plants which became apparent after the ice sheet in early 1937 is somewhat uncertain. Two plants were observed in the late summer of 1936 which showed unmistakable symptoms of bacterial wilt. While no plants were dug, a survey of the plot at that time failed to reveal any more diseased individuals. Considering that the planting was only in its second year of growth, it is unlikely that the disease had progressed very far even though many individuals may have become slightly infected before the winter of 1936–37. Because the one wilt-resistant strain included in the test, No. 15, was most severely damaged (96-percent mortality) while wilt-susceptible strains were damaged as little as 12 percent, it appears to be a safe assumption that wilt was not a major cause of death on the experimental plot.

MORTALITY ON THE EXPERIMENTAL PLOT

Counts were made May 8 and 9, 1937, of the living and the dead plants on the experimental plot. The position of the dead individuals was clearly marked by stem residues, as growth during the previous fall had been abundant. Classification was made without difficulty for the most part. Between the plants which had been killed outright and those which were clearly in a condition to survive, however, there was a small group less easy to place. These plants bore one or more living, but weak, shoots and obviously had suffered severe injury. If it appeared that such a plant was more likely to die than to survive, it was entered in the "dead" class; otherwise it was counted as living.

The total number of plants and the percentage killed have been collected according to strain and range in table 2. Considerable variation is noted between the mean values. Strain 3 is the Grimm check, in which 42 percent of the plants were killed, on the average. Hardigan, the other check variety, is strain 9 and showed 47.5 percent mortality. The remaining strains range from 12 percent to 96 percent killing, the general mean for all strains being 42 percent.

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TABLE 2.—*Mortality in alfalfa strains on experimental plot at Madison, Wis.*

Strain no.	Total plants and percentage killed in range—												Total plants	Mean percentage of dead plants
	I		II		III		IV		V		VI			
	Total plants	Killed	Total plants	Killed	Total plants	Killed	Total plants	Killed	Total plants	Killed	Total plants	Killed		
	Number	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*
1	25	60	26	100	25	20	23	17	25	28	26	15	150	40.00
2	24	100	24	50	25	44	25	8	24	4	26	38	148	40.67
3	25	72	25	40	21	52	24	17	24	29	24	42	143	42.00
4	25	44	21	67	24	17	24	17	25	48	15	40	134	38.83
5	25	4	25	60	24	63	25	56	24	38	26	38	149	43.17
6	25	16	25	16	23	39	24	25	25	64	23	13	145	28.83
7	25	88	25	40	25	12	23	61	25	8	22	14	145	37.17
8	25	20	25	12	25	16	23	17	24	4	25	8	147	12.83
9	25	76	24	58	24	33	24	4	25	72	24	42	146	47.50
10	26	31	21	90	25	28	24	21	25	68	26	12	147	41.67
11	22	82	26	15	25	0	24	17	25	8	25	4	147	21.00
12	25	92	25	32	25	52	25	72	25	48	25	8	150	50.67
13	22	14	25	40	26	46	25	8	25	12	25	4	148	20.67
14	25	60	25	36	25	76	24	46	25	80	25	28	149	54.33
15	25	100	24	96	25	100	24	100	23	100	25	80	146	96.00
16	26	96	23	87	25	12	26	27	25	68	24	4	149	49.00
17	27	70	25	20	24	33	25	8	25	64	26	4	152	33.17
18	23	91	23	96	16	100	24	54	24	88	23	52	133	80.17
19	26	31	25	60	25	8	23	48	25	36	26	8	150	31.83
20	24	71	25	44	24	8	25	48	25	36	25	12	148	36.50
21	26	96	25	52	26	54	25	68	25	64	25	40	152	62.33
22	25	56	23	70	24	25	25	40	25	24	25	24	147	39.83
23	25	76	26	69	24	88	23	84	25	28	26	35	151	63.33
24	23	96	25	4	24	21	25	12	23	30	26	19	146	30.33
25	26	62	25	36	26	73	25	32	25	28	26	42	153	45.50
26	23	30	23	39	23	0	24	75	25	92	24	33	142	44.83
27	26	58	23	78	25	84	25	92	24	63	26	54	149	71.50
28	25	52	25	68	21	100	24	75	24	83	25	8	144	64.33
29	25	16	25	8	25	12	25	24	25	8	25	4	150	12.00
30	25	16	25	68	26	19	25	8	24	29	26	23	151	27.17
31	27	63	24	0	24	21	25	4	24	25	24	0	148	18.83
32	27	44	25	4	25	56	25	48	25	32	26	58	153	40.33
33	24	29	24	13	23	35	25	20	25	12	25	40	146	24.83
34	25	32	23	30	20	30	22	36	24	29	25	44	139	33.50
35	26	31	25	92	25	48	24	88	24	33	23	48	147	56.67
36	25	4	25	24	25	48	25	16	23	70	26	23	149	30.83
37	23	48	25	44	24	46	25	12	24	17	25	40	146	34.50
38	21	86	21	95	22	73	22	86	24	71	24	42	134	75.50
39	26	31	24	63	25	16	25	24	24	21	26	4	150	26.50
40	26	19	24	21	25	40	24	8	24	8	25	8	148	17.33
41	26	81	24	4	25	20	26	58	24	21	23	9	148	32.17
42	25	100	25	76	25	76	25	52	24	58	26	38	150	66.67

The first question to be considered is whether the strains differ significantly in survival. Proceeding according to the analysis of variance technique customary with a Latin-square arrangement, the variation in mortality about the general mean, as measured by sum of squared deviations, has been broken down into four components with their associated degrees of freedom as shown in table 3.[5] If the

[5] The variance of an observed proportion P' is known to be $P(1-P)/N$, where P is the theoretical proportion which it samples and N is the number of observations upon which it is based. Consequently if the data in an experiment are expressed in percentages, and if the percentages differ widely among themselves—suggesting differences in the values of P sampled—then they may be of widely differing accuracy as measured by their variances. One of the assumptions underlying the analysis of variance technique is that the observations of the various classes are distributed about their true mean values, equal or different, with the same variance. Clearly this fundamental assumption is violated when percentages are used if differing mean values are admissible, owing to column and range effects. The extent to which error has been introduced can be determined by transforming the percentages to some new variable which will have a constant or nearly constant variance over the entire set of data at hand. It can be shown mathematically that the appropriate transformation is to the new variable θ defined by $\theta = \text{arc sin}\sqrt{P'}$, and this has been used by Bartlett (*1*, *2*), Bliss (*3*, *4*), and Cochran (*5*), in the papers cited below. The present analysis was repeated in these new units. It will be sufficient to indicate here the F values obtained in this analysis—they have the same respective significance levels. They are 12.18, 19.61, and 6.52 for ranges, columns, and strains, respectively. Clearly one can draw the same inferences from these as from the original values; it is quite remarkable that the changes have been so slight.

variation over the field is such as would arise through random sampling from a population in which column, range, and strain effects are nonexistent—the null hypothesis being postulated—and if the Latin-square type of analysis were exactly applicable, then the mean squares in column 4 in table 3 would have the same expectations. Actually the residual mean square, 532.94, is biased to some extent, as was mentioned in the footnote on page 64, the exact extent of the bias being unknown. From Goulden's assessment, however, it appears that this type of bias is generally positive so that the value 332.94 is probably too large. Consequently, if the mean square between strains is significantly larger than 332.94, it is probably significantly larger than the true, but unknown, estimate of residual variance Similarly for the other comparisons.

TABLE 3.—*Analysis of variance for percentage of alfalfa plants killed on experimental plot at Madison, Wis., during the winter of 1936–37*

Variance due to—	Degrees of freedom	Sum of squares	Mean square	*F*	1 percent [1] values
Ranges	5	20,391	407.20	12.25	3.11
Columns	5	32,350	6470.00	19.43	3.11
Strains	41	86,093	2099.83	6.31	1.28
Residual	200	66,587	322.94		
Total	251	205,421			

[1] Snedecor (*8, pp. 174–177*).

As was shown in the column labeled "*F*" (table 3), the mean square between strains is over six times the residual mean square. Comparing this with the 1 percent value of *F*, 1.28, given in the last column of table 3, the existence of strain effects is strongly suggested, since the mean square for "between strains" would exceed the residual mean square about once in 100 trials were the null hypothesis true. In brief, the observed event is highly improbable on assumption of the null hypothesis; therefore if the latter be rejected on this evidence, there is only 1 chance in 100 that it is rejected falsely. In this sense the analysis evidences the existence of differences between strains.

In like manner the analysis indicates the existence of differences between columns, and between ranges. It should be noted in this connection that when the significance of one effect is demonstrated this does not invalidate the test for another. This is an important property of the analysis of variance technique.

Since the Latin-square type of analysis is not strictly applicable to the lay-out at hand, it is of interest to inquire how the variability arising from strain differences and residual error, as measured by the between strain mean squares, compares with that arising from all sources other than between strain differences. To do this the sums of squares for ranges and columns are combined with that labeled "residual" in table 3, and the total divided by 210 degrees of freedom, yielding a mean square of 568.70. Comparing the between strains mean square with this, *F* is found to be 3.69, a value significant at the 1-percent level of significance. In other words, the evidence strongly supports the conclusion that there are significant differences in mortality between strains.

One may next inquire which comparisons are significantly different. Accepting the respective means as the best estimates of mortality for the strains concerned, it is desired to determine what difference between means is needed in order that it may be adjudged significant at a stated level of significance. This difference is calculated as follows:

$$(\bar{x}'-\bar{x}'')_{percent}=t_{percent}\times S\sqrt{\frac{1}{N_1}+\frac{1}{N_2}}$$

in which $(\bar{x}'-\bar{x}'')_{percent}$ is the magnitude of a difference necessary for significance at the level of significance (e. g., 5 percent, 1 percent) chosen, $t_{percent}$ being the tabulated value for 200 degrees of freedom and the particular level of significance (i. e., percent desired, S the square root of the residual mean square in table 3, and $N_1=N_2=6$. In this manner the 5 percent and 1 percent minimal significant differences were obtained and were found to be

$$(\bar{x}'-\bar{x}'')_{0.05}=20.74 \text{ percent and } (\bar{x}'-\bar{x}'')_{0.01}=27.25 \text{ percent}$$

The mean percentage of plants killed in Grimm (strain 3), a standard variety for the section, is 42.0, as shown in table 2. The others may be conveniently compared with it. The upper and lower bounds for significant difference from Grimm are as follows: For a 5-percent level of significance, upper bound 62.7, lower bound 21.3; and for a 1-percent level of significance, 69.2 and 14.7, respectively. This means that any strain in which the mean percentage of killing is either at least 62.7, or not more than 21.3, may be considered to be significantly different from Grimm at the 5-percent level of significance. Similar deductions may be made for the 1-percent values.

The positions of the 41 other strains in relation to that of the Grimm variety are depicted in figure 4, in which the limits of significance, according to the above criteria, are indicated. The differences in the amount of killing shown between most of the strains and Grimm are no larger than might frequently be expected to result from random sampling alone. There are several strains, however, some of which are highly susceptible to ice-sheet injury, others relatively resistant, which appear to differ significantly in this respect from the Grimm variety. The occurrence of strains which appear to be able to withstand ice better than this standard commercial type is of particular interest. There is little room for doubt that nos. 8 and 29 which showed 12.8 percent and 12.0 percent killing, respectively, are in the latter group; strains 11, 13, 31, and 40, showing 21.0, 20.7, 18.8 and 17.3 percent, respectively, are very probably so.

SURVIVAL OF INBRED AND HYBRID STRAINS

The average amount of killing in seven inbreds, based on six observations each, is 44.4 percent. The average percentage of killing in the 17 hybrids into which these inbreds enter, based on six observations each, is 37.4. The difference is probably significant, the calculated value of t [6] being 2.10 whereas the tabulated value for 200 degrees of freedom is 1.97 for the 5-percent level of significance and 2.60 for the 1-percent level. It is a reasonably safe conclusion, there-

[6] t is computed from a simple rearrangement of the formula on this page, but in the present instance $N_2=42$, $N_2=102$, and $(\bar{x}'-\bar{x}'')$ is the observed difference between the two means.

fore, from the data at hand, that hybrid strains in general survive better than inbred strains, the magnitude of the difference being approximately 7.0 percent.

DISCUSSION

The data which have been presented show that, under the conditions of the experiment, hereditary differences between strains were important in determining survival under an ice sheet. This finding is the more significant because the stocks under test were relatively

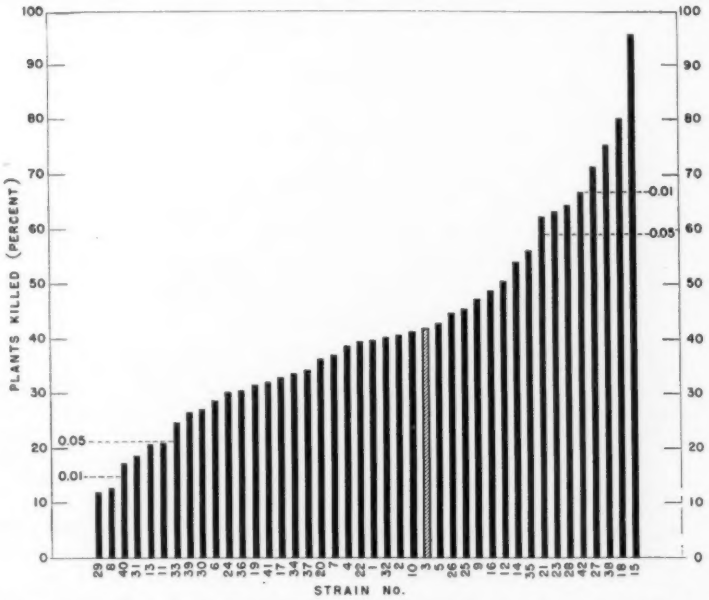

FIGURE 4.—The 42 strains of alfalfa on the experimental plot at Madison, Wis., arranged in ascending order of percentage of plants killed by the ice sheet. The Grimm check variety is represented by the crosshatched bar. The upper and lower bounds demarking percentages of killing significantly different, statistically, from the value for Grimm are shown for the 0.05 and 0.01 levels.

homogeneous and, with one exception, were derived from varieties considered suitable for the region. The existence in alfalfa of heritable differences of this kind is an interesting and significant fact in itself, but interpretation of the results in terms which have meaning for the plant breeder requires that another factor be taken into consideration, namely, the level of the test conditions. The latter statement is predicated on the hypothesis that as the ice-sheet factor becomes more severe genetic differences in ability to survive are overshadowed and may even disappear. Observation of ice-sheet injury in commercial fields supports this view, and indicates also that such levels of injury are frequently reached in the Great Lakes region.

It has been possible to show that the level of ice-sheet injury in the vicinity of the experimental plot was rather low relative to that in

nearby parts of the State where very severe losses of commercial acreage were experienced. This does not mean that on the test plot much damage was not done. On the contrary, nearly one-half the plants of the Grimm and Hardigan check varieties were killed and the remainder more or less severely injured. But the fact is established that ice-sheet conditions occur which impose a much more rigid test on the alfalfa plant's ability to survive than the one obtaining in the present experiment. The conclusion at which we arrive, therefore, must be a restricted one, namely, that there are observable significant differences between strains in ability to withstand a moderately severe ice sheet. What happens above this level remains for future experiments to determine.

If genetic differences in capacity to survive are overridden under the more destructive ice sheets, it is worth while to point out the biological implications of the fact. Adaptation, among other things, implies the possession of heredity which enables the organism to withstand the adverse elements in its environment. The extent to which the action of an unfavorable external agent is unopposed by the genotype is a measure of the organism's lack of adaptation in that particular respect. If, therefore, the effect on alfalfa of the more severe ice sheets is determined largely by external conditions, heredity playing a minor role, it merely means that the plant is correspondingly unadapted in this regard.

SUMMARY

Ice sheets, which form on the ground when rain falls at freezing temperatures, frequently cause great damage to alfalfa in Wisconsin and adjacent states. Very extensive damage resulted from an ice sheet during January and February of 1937 which, however, provided an unusual opportunity to study the reaction of alfalfa to this type of injury in an experimental plot containing 42 strains, located at Madison, Wis. Madison was in a transition zone between the severely damaged area to the south and east where the ground was bare, and the moderately damaged area to the north and west where the ground was covered by a protective layer of snow when the ice formed.

Many of the 42 strains were either self-fertilized selections from Grimm, or their hybrids. The parental lines had been selected on the basis of their cold resistance as measured by an artificial freezing test. Both the Grimm and Hardigan varieties were included as checks. The average mortality on the experimental plot was 42 percent, but values for individual strains ranged from 12 to 96 percent. A statistical study of the data indicates that significant differences in survival between strains occurred and, furthermore, that several strains were more resistant than either Grimm or Hardigan. Strains of hybrid origin were more resistant than their inbred parents.

The reaction of alfalfa strains to an ice sheet is dependent upon the severity of the ice-sheet complex. In the present instance the conditions imposed upon the experimental plot were mild as compared with those applying to a large part of southeastern Wisconsin. Under the more severe conditions significant differences established in the present study would no doubt be obliterated.

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TRANSMISSION OF SUGARCANE MOSAIC BY APHIDS [1]

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INTRODUCTION

The spread of sugarcane mosaic in the field frequently is difficult to explain on the basis of transmission by the corn leaf aphid (*Aphis maidis* Fitch) alone. It seems likely that other insects play a part in the spread of the disease. Since Brandes (*2*) [2] presented evidence showing that *A. maidis* could act as a vector of sugarcane mosaic, the problem of insects in relation to the transmission of the disease has been studied by a number of investigators. Kunkel (*6, 7*) published the results of studies in Hawaii which further showed the ability of *A. maidis* to transmit sugarcane mosaic. Chardon and Veve (*3*) conducted experiments in Puerto Rico which convincingly demonstrated that *A. maidis* could transmit sugarcane mosaic under field conditions. They also reported an experiment involving six plants in which four transmissions were obtained with a then unidentified species of the aphid genus *Carolinaia*. This experiment was not accompanied by controls, and it is possible that the aphid involved was *Carolinaia cyperi* Ainslie. Ingram and Summers (*5*), in work conducted in Louisiana during the years 1933–35, found that sugarcane mosaic could be transmitted from diseased to healthy plants by the rusty plum aphid (*Hysteroneura setariae* (Thomas)).

A number of different species of aphids were used by the writers in transmission experiments. Two other than *Aphis maidis* definitely were found to act as vectors of sugarcane mosaic and some evidence was obtained to indicate that still other species might be involved. The results of these studies are presented herein.

TRANSMISSION EXPERIMENTS

INSECTS USED

With the exception of a small number of miscellaneous tests, which are discussed in a subsequent section of this paper, the transmission experiments were confined to four species of aphids—*Carolinaia cyperi, Hysteroneura setariae, Sipha flava* (Forbes) (the yellow cane aphid), and *Aphis maidis*—the last-named species being used primarily to serve as a control in experiments involving other species.

A stock supply of aphids was obtained by placing large numbers of individuals, collected in the field, on plants growing in cages and then, after vigorous colonies had become established, transferring a single female to a fresh plant to start a colony from which individuals could be taken for use. By this procedure a homogeneous stock of aphids was obtained and used in all the experiments.

[1] Received for publication February 11, 1939. These studies were conducted in cooperation with the Puerto Rico Experiment Station, U. S. Department of Agriculture, at Mayaguez, P. R., where various facilities, including office and laboratory space and field plots, were furnished.

[2] Italic numbers in parentheses refer to Literature Cited, p. 78.

OTHER MATERIALS

The original stock of virus used in these experiments was obtained by selecting a single plant showing well-marked mosaic symptoms from a group of plants grown from seed pieces from diseased plants. All subsequent inoculations were made either from this plant or from other plants infected in serial transfers from the same source. All diseased plants were grown in porous clay pots.

The experimental plants were grown both from cuttings and from seed, in either porous clay pots or wooden flats having a soil depth of from 6 to 8 inches. In the case of cuttings some of each of the two sugarcane (*Saccharum officinarum*) varieties SC 12 (4) and BH 10 (12) were used, but the seedlings were grown exclusively from seed from the variety SC 12 (4). The temperature of the growing house averaged about 85° F.

Small tube cages, ranging from 2 to 3½ inches in diameter and from 6 to 14 inches in height and made of celluloid, were used for confining the insects on healthy sugarcane plants. The cage was placed over the entire plant. Aphids were then placed on the plant, and the upper end of the cage was covered with cheesecloth held in position by a rubber band.

METHOD OF HANDLING INSECTS

Transfer of the insects from one plant to another was accomplished by means of either a camel's-hair brush or an aspirator similar to the type described by Kunkel (*6*) for handling leafhoppers. In each test large numbers of aphids were confined on a diseased plant and then, at the time of making transfers to healthy plants, only those individuals that were observed to be feeding were selected. During the early part of the work from 15 to 20 aphids were confined on an experimental plant, but the number later was increased to 50. The aphids were confined on a diseased plant from 24 to 48 hours and then transferred to a healthy test plant for a similar period. At the end of that time the cages were removed and the plants carefully sprayed with a strong solution of pyrethrum and soap, after which they were transferred to the growing house.

The walls of the growing house, as well as all outdoor cages, were covered with 30-mesh copper screen, and the roof was covered with Celloglass. As a protection against ants or other insects the growing house and outdoor cages were surrounded by moats which were kept filled with either water or oil.

After exposure to infective aphids the experimental plants were examined daily for 30 to 40 days for evidence of mosaic. Plants showing no symptoms up to this time were observed at less frequent intervals for an additional 30 days before being discarded. At all times the number of control plants was equal to the number of exposed plants. Throughout the course of the tests all plants in the growing house were carefully sprayed with a strong solution of pyrethrum and soap at 3- or 4-day intervals. Sugarcane plants suffered no apparent injury as a result of the pyrethrum-soap spray, but nicotine sulphate, which was originally used, caused severe burning.

EXPERIMENT WITH CAROLINAIA CYPERI

Colonies of *Carolinaia cyperi* needed for experimental purposes were reared on the sedge *Cyperus rotundus* in screened cages. When confined on sugarcane plants, particularly on young seedlings, the aphids fed readily during the first 12 to 18 hours, after which they became restless and crawled about over the plants and the sides of the cages. Large numbers, however, survived for 3 or 4 days and some were still alive at the end of 5 or 6 days.

In 30 tests a total of 192 healthy plants were exposed to *Carolinaia cyperi* taken from diseased plants, and 60 of these, or 31.3 percent, developed mosaic. Seventy-three of the plants were grown from cuttings, and 11, or 15 percent, of these became diseased. Of the remaining 119 plants, all of which were grown from seed, 49, or 41.2 percent, became diseased.

The incubation period in plants grown from cuttings ranged from 20 to 42 days, with an average of 27.6 days. In seedlings the range was from 7 to 28 days, with an average of 18.2 days.

EXPERIMENT WITH HYSTERONEURA SETARIAE

In Puerto Rico one of the preferred hosts of *Hysteroneura setariae* is goosegrass (*Eleusine indica*), and since this grass grows well in cages, it was selected as the host plant for rearing the stock supply of this aphid.

One hundred and thirty-seven plants were exposed in 26 tests to infective *Hysteroneura setariae*, and 11, or 8 percent, developed mosaic. Forty-five of the plants were grown from cuttings, and 1 became diseased; 92 were grown from seed, and 10, or 10.9 percent, became diseased.

It will be noted that the percentage of successful transfers with *Hysteroneura setariae* is comparatively low, but the results are considered sufficiently reliable to justify the conclusion that this aphid can act as a vector of sugarcane mosaic. It is evident, however, that it was considerably less efficient as a vector of sugarcane mosaic than either *Carolinaia cyperi* or, as will be shown later, *Aphis maidis*. The results with the latter are in agreement with those obtained by Ingram and Summers (5).

EXPERIMENT WITH SIPHA FLAVA

The technique employed with *Sipha flava* differed from that used with the other species of aphids in that the stock supply was maintained continuously on mosaic-infected sugarcane by means of successive transfers to diseased plants. From this colony aphids were transferred directly to healthy plants. In 9 tests 75 healthy plants, 35 from cuttings and 40 from seedlings, were exposed to *S. flava* taken from diseased plants. None of these developed mosaic symptoms during the 2½ months that they were kept under observation. When 20 to 50 individuals of *S. flava* were confined on a young seedling for 2 days or more, the older leaves usually developed a reddish-brown color, the characteristic injury associated with this species of aphid, and gradually died. Somewhat similar but considerably less severe symptoms resulted when *S. flava* was confined on young plants grown from cuttings.

EXPERIMENT WITH APHIS MAIDIS

Several plants were tried in seeking a host for maintaining a constant supply of *Aphis maidis* in small cages, and dwarf popcorn (*Zea mays* var. *everta*) was found to be the most satisfactory.

Infective individuals of *Aphis maidis* were confined on 200 healthy sugarcane plants and 69 of the plants, or an average of 34.5 percent, developed mosaic. Of this number 57 were grown from cuttings, and 19, or an average of 33.3 percent, became diseased. The remaining 143 exposures were made on seedlings and 50, or 35.0 percent, of these were infected by transfer of the virus.

EXPERIMENTS WITH OTHER APHIDS

In February and March 1936 some miscellaneous experiments were conducted with three species of aphids that were available on plants growing near the insectary at Mayaguez, all of which were grown from seed. Nine plants were exposed to *Aphis rumicis* L., nine to *Macrosiphum rudbeckiae* (Fitch), and four to *A. nerii* Fonsc. The only plant to become diseased was one in the series exposed to *A. nerii*. Although it is realized that these tests were by no means extensive enough to give conclusive evidence, the implications are of considerable interest.

OCCURRENCE OF THE EXPERIMENTAL APHIDS IN PUERTO RICO

According to available records, *Carolinaia cyperi* is limited in its distribution to tropical and subtropical climates and, so far as known, its host-plant range is confined to certain sedges of the genus *Cyperus*. *C. cyperi* was originally described by Ainslie (*1*) from specimens collected at Lakeland, Fla., on *Cyperus esculentus*, the common nutgrass, or chufa, of the Southern States. The writers found *C. cyperi* to be quite generally distributed in Puerto Rico, where it appears to colonize only on "coqui," (*Cyperus rotundus*). Coqui constitutes one of the more serious weed pests in Puerto Rico and seems to thrive during favorable seasons on many types of soil. Records taken in the vicinity of Mayaguez, which covered the period from September 1935 to July 1936, showed that this aphid attained its greatest abundance between the extremely wet and the extremely dry season. Coqui grows abundantly in and around sugarcane fields, and during the process of cultivation and otherwise caring for sugarcane plantings it is destroyed, thus forcing the aphids to migrate in search of new host plants.

During certain seasons of the year *Hysteroneura setariae* was found in considerable abundance on a number of grasses growing in the vicinity of cane fields. In some localities in Puerto Rico, particularly along the central southern coast, *H. setariae* has been found to colonize on cane, and fairly heavy local infestations occasionally were observed. The colonies were practically always found at the junction of the leaf blade and sheath.

Sipha flava is present in Puerto Rico at all seasons of the year, but according to records it is most abundant during the dry season and in the more arid regions. In the section of Puerto Rico having comparatively little rainfall, which, generally speaking, includes the south side of the island, *S. flava* is a pest of considerable importance through-

out the year, but the greatest damage occurs on young sugarcane during the spring months.

Aphis maidis is not known to feed on sugarcane to any appreciable extent in Puerto Rico. In rare instances small colonies have been observed to survive for a few weeks on small sugarcane plants in cages. In the transmission studies it was found that a few *A. maidis* individuals would survive on sugarcane plants for a week or 10 days, but the majority died within 4 to 6 days.

DISCUSSION OF EXPERIMENTS

The results of experiments presented in this paper clearly demonstrate that the aphid *Carolinaia cyperi* can transmit mosaic from diseased to healthy plants. The aphid *Hysteroneura setariae* also was shown to be a vector of sugarcane mosaic, thus confirming the results obtained by Ingram and Summers (*5*) in Louisiana. These findings will undoubtedly help to explain the field spread of sugarcane mosaic, particularly under such conditions as exist in Puerto Rico.

Although *Carolinaia cyperi* does not colonize on sugarcane, its host plant *Cyperus rotundus* commonly grows in and around sugarcane fields, and it does not seem improbable that during the course of migration, resulting from such factors as overcrowding or destruction of its host plant, it may feed for short intervals on sugarcane and thus become a means of spreading mosaic.

When seedlings were used as the experimental plants, *Carolinaia cyperi* proved to be as good as, if not better than, *Aphis maidis* as a vector of mosaic, but when cuttings were used the percentage of successful transfers of the disease was considerably less than with *A. maidis*. This probably was partly attributable to the fact that *C. cyperi* fed less readily on plants grown from cuttings than on seedlings, possibly because of their comparative softness and tenderness and also because of their grasslike characteristics in the early stage of growth. *Hysteroneura setariae* proved to be a less efficient vector of sugarcane mosaic than *A. maidis* a finding which confirms the results obtained by Ingram and Summers (*5*).

Inasmuch as the proved vectors of sugarcane mosaic are not known to occur on sugarcane to any appreciable extent in Puerto Rico, with the exception of localized infestations of *Hysteroneura setariae*, it appears that field dissemination is largely dependent upon insects that are more or less incidentally associated with sugarcane. Evidence in support of such a contention has been presented in connection with certain other viruses. Drake, Tate, and Harris (*4*) found that aphids, which do not normally feed on the onion plant, are responsible for the natural spread of yellow dwarf, a virus disease of onions. Zaumeyer and Kearns (*8*) succeeded in transmitting bean mosaic by means of 11 species of aphids, none of which ordinarily colonize on the bean plant, and these workers concluded that other species probably would be found to act as vectors of this disease.

The percentage of successful transfers obtained in insect-transmission experiments with sugarcane mosaic has been comparatively low, and it has been necessary to use large numbers of plants to obtain conclusive results. Such a condition necessitates the use of considerable greenhouse space, which is expensive and frequently not available, for growing the plants during the observation period.

In an effort to simplify the situation, plants grown from seed of the variety SC 12 (4) were used and the results compared with those obtained from plants grown from cuttings.

Seedlings proved to have an advantage from the standpoint of convenience and rapidity of handling and economy of greenhouse space. In 1 square foot of greenhouse space 16 to 20 seedlings could be conveniently used in transmission experiments, whereas in a similar space not more than 6 to 8 plants grown from cuttings could be satisfactorily handled. Of 262 seedling plants exposed to *Carolinaia cyperi* and *Aphis maidis*, 99, or an average of 37.8 percent, developed mosaic. From 130 exposures of plants grown from cuttings 30, or 23 percent, resulted in positive transmissions. In seedlings the average incubation period of the disease in the plant was 19.3 days, and in cuttings it was 26.2 days.

The chief disadvantage in using seedlings was that plants in a satisfactory stage of growth for conducting small cage experiments were not available throughout the year.

SUMMARY

Experiments are described which demonstrate that the aphid *Carolinaia cyperi* can transmit mosaic from diseased to healthy sugarcane plants. Out of 192 plants exposed to *C. cyperi* taken from diseased plants, 60, or 31.2 percent, became diseased, and of 200 healthy plants exposed to infective *Aphis maidis*, 69, or 34.5 percent, developed mosaic.

Field observations showed that *Carolinaia cyperi* was present on its host plant, *Cyperus rotundus*, in and around sugarcane fields in Puerto Rico in considerable abundance during certain periods of the year.

Transmission experiments with *Hysteroneura setariae* convincingly demonstrated that this aphid could transmit mosaic from diseased to healthy sugarcane plants, thus confirming the evidence obtained by previous investigators.

Of a total of 75 healthy sugarcane plants exposed to *Sipha flava* taken from diseased plants, none developed mosaic.

In most cases a considerably higher percentage of transmission was obtained when seedling plants were exposed to aphids that had fed on diseased plants than when cuttings were used.

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